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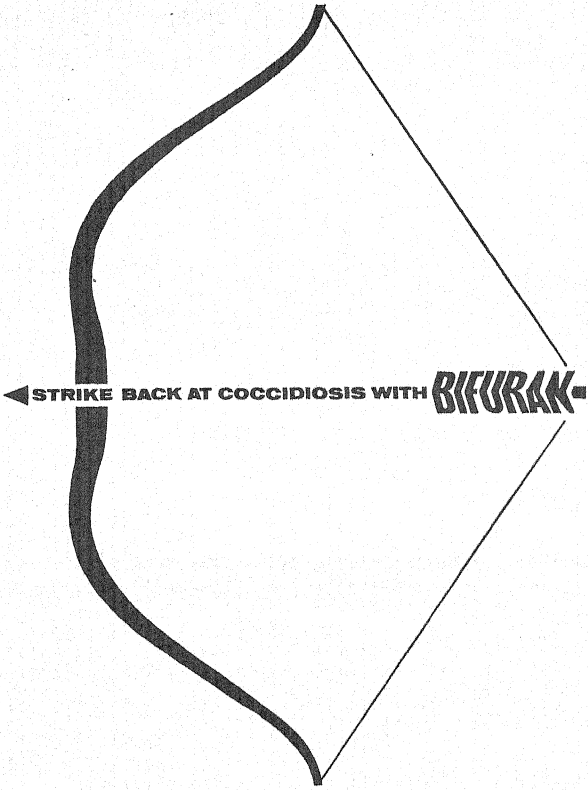

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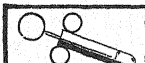

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INVESTIGATION OF AFRICAN HORSE-SICKNESS IN INDIA

I. STUDY OF THE NATURAL DISEASE AND THE VIRUS

KEERTI V. SHAH

*Virus Research Centre, Poona**

(With the technical assistance of B. G. Patil and S. K. Virkar)

Received: July 10, 1963

AFRICAN horse-sickness (AHS) as a clinical entity has been known in South Africa from as early as the 18th century (Wooldridge, 1934). Arnold Theiler transmitted the disease from sick to healthy horses by inoculation of bacteria-free filtrates of blood or serum and thus established its viral etiology. By clinical observations and infection-challenge experiments in horses he recognised the existence of antigenic differences between strains. He suggested on epidemiological grounds that the disease was in all probability arthropod transmitted and postulated a non-equine 'reservoir' for the virus. He also developed methods of immunisation for horses and mules (Theiler, 1930).

A major technical advance was made when Alexander (1933), following Max Theiler's demonstration that mice were susceptible to yellow fever virus by the intracerebral route (Theiler, 1930), adapted the AHS virus to mouse brain. By intracerebral neutralization tests in mice he confirmed and enlarged Arnold Theller's observation that there were several antigenic types of AHS virus. He (1935) demonstrated that serial intracerebral passage in mice led to reduction of virulence of the virus for horses, and developed the live polyvalent avirulent virus vaccine in current use. Du Toit (1944) considered that *Culicoides* midges were the vectors. This was confirmed subsequently by numerous isolations from *Culicoides* collected during epizootics in South Africa as well as by feeding wild caught *Culicoides*, first on a sick horse and thereafter on a susceptible horse which developed the disease (Du Toit, 1960). McIntosh (1958), who studied 42 mouse-adapted strains, divided them into seven antigenic types separable from each other by the absence of cross protection in serum dilution neutralization tests with hyperimmune rabbit sera. He (1956) also showed that the different antigenic types gave a common group reaction in complement fixation (CF) test and were not distinguishable by that technique. Recently, Pavri (1961) demonstrated the capacity of AHS virus to agglutinate equine erythrocytes.

The earliest clinical diagnosis of African horse-sickness in India was made in late April, 1960, in a cavalry unit in Jaipur. The onset of the first case was on April 21, 1960, and in a period of six weeks 107 of the 323 horses and none of the four mules in the unit were affected. Of these, 36 horses died—all within ten days of onset. The average length of illness in the fatal cases was 4·5 days, and 25 animals died within five

*The Virus Research Centre is jointly maintained by the Indian Council of Medical Research and the Rockefeller Foundation.

days. Twenty-three horses recovered. Forty-eight animals were sacrificed, 24 during convalescence (16 or more days after onset) and 24 within ten days of onset. Subsequently, the disease spread to 12 states of the Indian Union claiming a mortality of over 16,000 horses (Sahai, 1961). The first virus strains were isolated by a team from the Indian Veterinary Research Institute, Mukteswar (U.P.).

This communication reports the virological and epidemiological investigations of the natural disease.

MATERIAL AND METHODS

Animals were bled from the jugular vein with sterile vacutainers for serum or with syringe and needle for whole blood. Whole blood was collected in heparin to give a concentration of 0·1-0·2 mg. of heparin per millilitre of blood. At autopsy organs were collected in sterile tubes without any preservatives. Specimens were transported to Poona on wet ice.

Infant (two- to four-day-old) and adult (three- to five-week-old) white mice were inoculated intracerebrally (IC) with 0·02 and 0·03 ml. of blood serum, or approximately 10 per cent tissue suspension. Passages were made with 10 per cent mouse brain suspensions in 0·75 per cent bovine albumin phosphate saline (BAPS). Strains were identified as AHS by testing 10 per cent saline extract of infected mouse brain against convalescent sera from naturally infected horses in a quick complement fixation test.

Complement fixing antibody titres of sera were determined on plastic plates by the method of Fulton and Dumbell with some modifications (Pavri *et al.*, 1962). Antigen for CF test was prepared by acetone ether or sucrose acetone extraction of infected infant mouse brains of strain 603395 (isolated from heparinised blood of a Jaipur horse and identified at Onderstepoort).

Neutralization (N) tests were performed with uninactivated sera. Quantitative estimations of antibodies were made either by test of undiluted serum against varying ten-fold dilutions of the virus or by testing five-fold dilutions of serum against a single virus dose (McIntosh, 1958). Dilutions of serum and virus were made in BAPS. Serum-virus mixtures were incubated at 37°C for two hours and then at 4°C overnight, and were inoculated IC into groups of six adult mice. End points were calculated by the method of Reed and Muench. Serum titres refer to the final serum dilution after addition of virus.

Qualitative neutralization tests were performed by test of undiluted serum against a single virus dose. The N test virus was the 8th, 9th or 10th adult mouse passages of strain 603400 (from spleen of an autopsied horse at Jaipur). The virus preparation employed in neutralization tests was the supernate of 20 per cent mouse brain suspension in 0·75 per cent BAPS after centrifugation at 5,000 r.p.m. for one hour at 4°C. The virus was stored shell frozen in sealed glass ampoules at —50°C. Interpretations of protective, partially protective and negative were made as follows: specific mortality ratios of 0/6, 1/6, 0/5, 1/5 or 0/4 protective; 6/6, 5/6, 5/5, 4/5 and 4/4 negative; and 2/6, 3/6, 4/6, 2/5 and 3/5 partially protective. Sera which prolonged the average survival time (AST) significantly (Mean AST + 3 S.D.) were interpreted as partially protective.

Test of susceptibility to desoxycholic acid (DCA) (Director of Veterinary Services, Onderstepoort, 1960; personal communication), was performed with strain 603395. Four preparations of the virus were tested in two experiments. Infected 10 per cent mouse brain suspensions of AHS as well as of a control virus known to be inactivated by DCA were centrifuged at the same time at 10,000 r.p.m. for one hour at +4°C. DCA in concentration of 1/500 was added to an equal quantity of 10^{-1} or 10^{-2} dilution of the AHS and control viruses. After incubation at 37°C for one hour the viruses were titrated. In one instance ten-fold dilutions of the virus were made first and DCA was added to each dilution prior to incubation.

RESULTS

Isolation of virus: Specimens of serum and heparinised blood taken simultaneously from an animal were tested on 41 occasions, yielding two isolations from serum and 14 from heparinised blood. The correlation between virus isolation from heparinised blood and serum taken at the same time is given below. Both serum and

		Heparinised blood		
		+ve	—ve	
Serum	+ve	2	0	2
	—ve	12	27	39
		14	27	41

heparinised blood were positive for virus isolation twice and negative 27 times. In 12 instances virus was isolated from heparinised blood but not from serum. When both serum and heparinised blood were positive the virus titres of the sera in infant mice (per 0·02 ml.) were $10^{-1\cdot5}$ and 'traces' as compared to $10^{-4\cdot6}$ and 10^{-1} of the corresponding specimens of heparinised blood. It was evident that heparinised blood was better than serum as a source for virus isolation.

The early field specimens were inoculated simultaneously in infant and adult mice, and ten isolations were made in adult as against 15 in infant mice (Table I). It was not clear if the failure of isolation in adult mice in five instances was due to a lower susceptibility of adult mice or to an insufficient period of observation (between 20 to 27 days for the above five specimens). The illness in infant mice differed from that in adult mice in several ways. In infant mice the shortest incubation period was four days and the longest 20 days as compared to 11 and 26 days in the adult. When both the infant and adult mice were inoculated simultaneously, the onset of illness in adults was on an average about eight days after that in infants. Sometimes the sickness in adults occurred as late as 13 to 17 days after that in infants. With a high concentration of virus, as with the blood of horse 87, all infant mice fell sick and died on the fourth day; the sickness in adults was first noticeable only after 11 days with a mortality of six out of seven by the 23rd day. The infant mice died within a day or two of the onset of illness; in adults the onset of illness in individual mice of a group was spread

over several weeks and the duration of illness was often as long as 10 to 30 days. Some of the first passage adult mice recovered from illness were subsequently resistant to intracerebral challenge by the virus.

Titre and duration of viremia: The reciprocal log LD_{50} titres for infant mice per 0·02 ml. of the positive heparinised bloods are indicated in Table I. They ranged from 'traces' (less than half mice died with undiluted blood) to $10^{-4.8}$ except for one specimen of one day of illness which titred $10^{-4.6}$.

TABLE I. COMPARISON OF HEPARINISED BLOOD AND SERUM FOR EFFICIENCY OF VIRUS ISOLATION IN INFANT AND ADULT MICE

Horse No.	Days after onset	Serum —10°				Heparinised blood —10°				Reciprocal log LD_{50} titre of heparinised blood in infant mice
		Adults		Infants		Adults		Infants		
		MR	Incubation period	MR	Incubation period	MR	Incubation period	MR	Incubation period	
77	2	0/5	..	0/7	..	1/5	12	4/8	10	1·0
87	1	8/8	16	4/6	9	7/7	11	7/7	4	4·6
95	7	0/8	..	0/7	..	3/8	19	2/5	20	Tr.
97	4	0/6	..	0/5	..	1/5	15	2/8	10	Tr.
126	7	0/6	..	0/8	..	8/8	17	8/8	11	0·4
178	2	0/8	..	0/8	..	0/7	..	3/6	17	Tr.
212	6	0/8	..	0/6*	..	8/8	18	5/5	9	1·0
277	2	0/8	..	0/6	..	2/7	19	6/8	8	0·3
278	2	0/8	..	0/7	..	3/7	24	5/7	7	1·0
264	2	0/8	..	0/8	..	0/7	..	7/7	8	1·2
317	8	0/5	..	0/7	..	0/6	..	2/6†	13	<1·0
383	9	0/8	..	0/6	..	2/8	14	4/5	10	1·2
465	2	No specimen				0/7	..	8/8	8	1·8
476	1	0/8	..	0/8	..	2/7	26	1/7†	13	0·5
29K9	7	0/8	..	0/7	..	0/8	..	5/6	14	0·9
Isolations		1/14		2/14		10/15		15/15		

*Virus from 10^{-1} dilution. Mouse of 9th day.
†From 10^{-1} dilution of specimen; 10^{-0} was unsatisfactory.
MR = Mortality ratio, adult mice frankly ill considered as dead for MR.
Tr. = Traces. Less than half mice died after inoculation of undiluted specimen.

Data derived from one or more specimens from 24 laboratory-confirmed cases of AHS were examined for frequency of virus isolation by day of onset (Table II).

Virus was present in blood till the ninth day after onset but not thereafter. Fifteen out of 21 specimens obtained in the first nine days yielded virus.

TABLE II. FREQUENCY OF VIRUS ISOLATION FROM HEPARINISED BLOOD AND SERUM BY DAY AFTER ONSET

Material	Days after onset									
	1	2	3	4	5	6	7	8	9	10
Heparinised blood	2/3*	6/6	..	1/2	..	1/2	3/3	1/2	1/3	0/3
Serum	1/3	0/5	..	0/3	..	1/4	0/5	0/2	0/4	0/3
Material	Days after onset									
	11	12	13	14	15	16–21	22–35	36–49	50–63	64–77
Heparinised blood	..	0/2	..	..	0/3	0/1	0/5	0/6	..	0/2
Serum	0/1	0/2	..	..	0/3	0/4	0/5	0/6	..	0/2

*Numerator indicates number positive for virus and denominator indicates number tested.

Identification of the virus: Sera from two convalescent horses from Jaipur reacted against the Jaipur virus isolates in quick complement fixation tests, an indication that the horses had undergone infection with the isolated virus type. In the beginning the specific diagnosis of AHS, however, could not be established at the VRC, as the type strains or specific immune sera were not available. Eight virus isolates (as second or third passage infant mouse brains) and two convalescent sera were, therefore, sent to the Onderstepoort Laboratories, South Africa. These were identified as strains of AHS virus by screening in CF tests against AHS immune serum. By serum dilution neutralization tests it was further shown that the eight Indian isolates were of one type and closely similar to or indistinguishable from the Pakistan virus. Test of convalescent sera of Jaipur horses against the seven-type strains revealed an immunological overlap between the Indian strain and types 3, 4, 5 and 6. The maximum overlap occurred with type 6 (Director of Veterinary Services, Onderstepoort, 1960; personal communication).

At the VRC a number of convalescent sera from Jaipur were tested against the Jaipur isolates and the Pakistan and type 6 viruses (the latter two strains were kindly supplied by the Director of Veterinary Services, Onderstepoort), testing dilutions of virus against undiluted serum. In five instances the isolate from the acute phase blood was tested against the convalescent serum from the same animal. The results are given in Table III. The neutralization indices of the convalescent sera against the Jaipur isolates were comparable to those against Pakistan and type 6 viruses. It was evident that the neutralization test with dilutions of virus and undiluted serum was not sensitive enough to detect the differences between antigenic types. The results of tests with dilutions of sera against a constant dose of virus are given in Table IV. The serum-antibody titres of the convalescent sera against the Indian and Pakistan viruses were comparable and were significantly higher than those against type 6 virus.

TABLE III. NEUTRALIZATION INDICES OF CONVALESCENT HORSE SERA AGAINST JAIPUR ISOLATES AND AGAINST PAKISTAN AND TYPE 6 AVIRULENT STRAIN

Strain	Titre	Passage level		Immune sera				Homologous serum	Other sera
		Infants	Adults	Horse 510 (42)	Horse 187 (39)	Horse 278 (40)	Horse 264 (40)		
Jaipur strains									
603389-1	5·2	0	5	±2·8	2·3	2·4	2·8	2·4	2·5, 2·2, 2·7
603393	5·3	1	5	†3·5	3·3	2·8	2·8	:	3·1, 3·0, †3·5, 2·7, 2·9
603400	6·1	0	6	3·1	3·3	3·6	3·4	:	3·0, 3·9, †4·1, 3·1, 4·1
603344	5·4	0	5	†3·8	3·4	†3·8	†3·9	†3·8	†3·7, 3·4, †3·8, 3·9
603338	5·6	0	6	†3·4	3·4	3·4	3·0	⩾3·6	3·1, ⩾4·1, ⩾3·6, 2·8
603350	5·0	1	5	†3·2	†3·5	†3·4	†3·2	:	†3·4, †3·5, †3·5, †3·1, †3·5
603374	4·7	0	6	†3·1	†2·9	†3·1	†2·9	:	2·7, †3·1, †3·0, †3·2
603365	4·7	1	3	†2·9	†3·2	†3·2	†3·2	:	†3·2, †3·2, †3·2, †3·2, †3·0
603380	±5·2	1	3	†3·7	†3·7	†3·7	†3·7	:	†3·7, †3·7, †3·7, †3·4, †3·7
603353	5·0	2	2	†3·5	†3·5	†3·5	†3·5	†3·5	†3·5, †3·5, †3·5, †3·5, †3·5
603359	5·5	3	2	†4·0	†4·0	†4·0	†4·0	†4·0	†4·0, †4·0, †4·0, †4·0, †4·0
Pakistan*	4·8	:		†3·2	2·8	†3·2	†3·2		
Type 6*	4·8	:	+100	†3·2	†3·2	†3·2	2·7		

Note: Figures in parentheses indicate day the post-infection.
*Received from South Africa.

†Read ⩾ before the figures.

TABLE IV. ANTIBODY TITRES OF CONVALESCENT HORSE SERA AGAINST THE INDIAN, PAKISTAN, AND TYPE 6 SOUTH AFRICAN VIRUS
(Dilution of serum against constant virus dose)

Serum No.	Days after onset	Indian virus 603400 2·3 log LD_{50}	Pakistan virus 604878 2·4 log LD_{50}	Type 6/604877 2·1 log LD_{50}
603339–4	42	1/160	1/96	1/14
603354–3	39	1/30	1/102	1/12
603372–3	40	1/26	1/104	1/10
603375–5	40	1/36	1/148	1/16
603366	35	1/50	1/64	1/14
603369	35	1/102	1/184	<1/10
603663–2	Non-immune	<1/10	<1/10	<1/10

Note: Test in adult mice intracerebrally.

In the Jaipur epidemic virus isolations were made from blood, serum or organs of 16 horses. In addition isolations from specimens submitted from other states were as follows: nine from Maharashtra State (six from West Khandesh and three from Poona) and eight from Uttar Pradesh (three from Allahabad and five from Mathura). In order to see if the strains from the different regions were antigenically alike, seven virus isolates from the three states were screened against two convalescent sera from Jaipur. Results are given in Table V. The antibody titres of the sera against the different strains were comparable. There was no evidence of any marked antigenic variations between the isolates in the three States.

TABLE V. ANTIBODY TITRES OF TWO JAIPUR CONVALESCENT SERA AGAINST STRAINS FROM THREE STATES

Locality	Strain	Passage	Log LD_{50} of virus	Titre of convalescent sera	
				603339–4	603369
Rajasthan					
Jaipur	603400	8	2·3	1/160	1/102
Maharashtra					
W. Khandesh	605058	3	2·4	1/28	1/112
W. Khandesh	605060	2	2·6	1/36	1/94
Poona	607862	3	2·5	1/40	1/60
Poona	607863	2	2·4	1/22	1/60
Uttar Pradesh					
Allahabad	606635	3	3·2	1/112	1/168
Mathura	606986–2	2	2·5	1/162	1/50

Development of CF and neutralizing antibodies to AHS virus: CF and neutralizing antibodies were detected very soon after the onset of illness, and it was not uncommon to isolate virus from heparinised blood, when sera collected at the same time had high levels of CF and neutralizing antibodies. In Table VI the CF and neutralizing antibody titres are given for sera of 14 horses obtained during the first nine days after onset, when heparinised blood taken at the same time yielded virus. Neutralizing antibodies were demonstrable in all 14 sera. CF antibodies were detected in eight, not detected in three and the results were equivocal in the remaining three sera. The three sera without CF antibodies had the lowest titres of neutralizing antibodies. In sera collected ten days or more after onset, high titres of CF and neutralizing antibodies were consistently found.

TABLE VI. CF AND NEUTRALIZING ANTIBODY TITRES TO AHS IN ACUTE SERA (Heparinised blood taken at the same time yielded virus)

Recorded day after onset	Serum No.	Reciprocal of CF titre	Neutralizing antibody titre (dose 1·8 log LD_{50})	Reciprocal log LD_{50} of virus titre in heparinised blood
1	603384-1	>64, 128	>1/1250	0·5
1	603393-1	<4, <4	<1/10*	4·6
2	603351-1	>64, 128	1/1250	1·0
2	603360-1	>64, 64	>1/1250	Traces
2	603363-1	8, 16	1/340	0·3
2	603372-1	<4, <4	<1/10*	1·0
2	603375-1	8, <4, <4	1/76	1·2
4	603336-1	32, 64	1/480	Traces
6	603387-1	<4, <4	1/14	1·0
7	603342-1	4, <4	1/400	0·4
7	603357-1	64, 32	>1/1250	Traces
7	603381-1	32, 64	>1/1250	0·9
8	603348-1	16, 4	1/186	<1·0
9	603390-1	<4, 4	1/382	1·2

*Sera protective when undiluted.

Extent of sub-clinical infection: At Jaipur the horses were under close clinical observation and daily temperatures were taken of all animals. Laboratory confirmation of diagnosis of AHS was made for all animals that survived the infection and for a number of those dying in the acute illness. This situation provided an opportunity to make an estimate of the frequency of sub-clinical infections.

There were 323 horses and four mules in the regiment, and 107 horses were clinically affected. At the end of the outbreak, 66 of the 216 unaffected horses and all four mules were bled and their sera examined in CF test. CF antibodies were detected in sera of two horses and one mule with titres of 1/64, 1/32 and 1/32 respectively. These animals were re-bled after five weeks and the positive serology confirmed. An estimate of the number of sub-clinical infections in the 216 healthy horses of the unit based on the examination of these 66 horses (with two positives) was seven. This gave the ratio of clinical to sub-clinical infection in the unit as 107:7 or about 15:1. It would seem that the overwhelming majority of AHS infections was overt.

Antibody survey of other animals: The results of neutralization tests of sera from different animal species in the AHS epizootic areas of Rajasthan and Maharashtra are given in Table VII. There is indication that donkeys and dogs probably became infected in nature. There was no evidence for infection of man, goats or birds. The single positive in 89 bovine sera may represent rare infection, non-specific neutralization or mislabelled serum.

TABLE VII. NEUTRALIZATION TEST RESULTS OF SURVEY SERA OF ANIMALS IN AFRICAN HORSE-SICKNESS EPIZOOTIC AREAS

Locality	Donor	Number tested	Positive	Partially protective	Negative
Rajasthan	Human	34			34
	Donkey	19			19
	Pony	1			1
	Sub-total	54			54
Maharashtra (1)	Buffalo	12			12
	Bullock	57	1	4	52
	Cow	11		3	8
	Goat	16			16
	Donkey	14	5	3	6
	Dog	6			6
	Bird	21			21
	Squirrel	1			1
	Sub-total	138	6	10	122
Maharashtra (2)	Cattle	9			9
	Sheep	2			2
	Goat	1			1
	Donkey	9			9
	Dog	14	9	1	4
	Sub-total	35	9	1	25
	Total	227	15	11	201

Note: All sera listed under Maharashtra (2) and the donkey sera from Rajasthan were submitted by the veterinary officers of the respective states for examination. Others were collected by the VRC staff. The human specimens were largely from the attendants of sick animals at Jaipur.

Behaviour of the virus in mice: Virus isolate 603395 from heparinised blood of a sick horse at Jaipur was maintained serially by intracerebral passage both in infant and adult mice; this afforded an opportunity to study the results of adaptation of virus to mice of different ages. In adult mice the incubation period shortened from 11 days in the first passage to three to five days by the fourth and the fifth passage. The mice died within one to three days of onset of illness and recovery from sickness did not occur. The adult mouse adapted virus when inoculated into infant mice had a shorter incubation period and comparable titre. The virus was non-pathogenic to infant and adult mice by the intraperitoneal route.

On continued intracerebral passage in infant mice, the incubation period for infant mice was reduced to one to two days and it was feasible to make a passage daily. However, after adaptation to infant mouse brain the pathogenicity of the virus altered for adult mice. The incubation period after inoculation of highest concentration of virus lengthened from three to four days to six to eight days, and was as long as 14 days after inoculation of small amounts of virus. There was a longer interval of two to seven days between onset of illness and death. Further, some of the mice inoculated with higher dilutions of virus fell sick and recovered, and were then resistant to intracerebral challenge with the virus. After 72 passages in infant mice the virus was passed in adult mice for ten passages without change in the pattern of long incubation period and prolonged average survival time (AST).

To illustrate this point the AST of adult mice inoculated with the infant and adult mouse adapted lines are compared for similar virus doses in Table VIII. The

TABLE VIII. COMPARISON, IN DAYS, OF AVERAGE SURVIVAL TIME OF ADULT MICE INOCULATED IC WITH INFANT MOUSE AND ADULT MOUSE PASSAGE 603395

Dose LD_{50}	Infant mouse strain		Adult mouse strain	
	Pass. 41	Pass. 50	Pass. 22	Pass. 27
10^{-1} to 10^{0}	14·0	14·0	7·0	10·0
10^{0} to 10^{1}	13·3	14·4	6·2	6·0
10^{1} to 10^{2}	11·2	10·4	5·8	5·2
10^{2} to 10^{3}	9·5	10·4	4·8	4·5
10^{3} to 10^{4}	8·8	10·6	4·2	4·3
10^{4} to 10^{5}	6·6	11·2	3·6	
10^{5} to 10^{6}	7·0	5·2		

AST (calculated for those mice that died) after inoculation of the infant mouse virus tended to be about twice as long as that after inoculation of the adult mouse virus. McIntosh (1958) also observed a similar change in the mortality pattern for adult mice after prolonged infant mouse passage. The titres of the two lines in infant and adult mice at different passage levels are shown in Table IX; contrary to the findings

of McIntosh (1958) there is no evidence of marked reduction in titre for adult mice by prolonged passage in infant mice.

TABLE IX. TITRES OF INFANT MOUSE AND ADULT MOUSE ADAPTED LINES OF 603395 IN INFANT AND ADULT MICE

		Reciprocal log LD_{50} titre	
		In infant mice (·02 ml.)	In adult mice (·03 ml.)
Infant mouse passage	41	6·8	6·9
	50	7·3	5·9*
	102	6·6	±6·5
Adult mouse passage	22	5·7	5·6
	27	4·3	4·9
	60	4·1	5·5

*Figure would be 6·6 if titre is calculated by mortality and/or morbidity.

Susceptibility of AHS virus to desoxycholic acid (DCA): In both the experiments the known susceptible control virus was completely inactivated by DCA and virus was not detectable even at the lowest dilution (Table X). The difference in titre between untreated and DCA-treated virus was, on both occasions, greater than 6·0 log LD_{50}. In contrast, the differences in titre between untreated and DCA-treated AHS virus were 0·8, 1·1, 0·8 and 1·6 log LD_{50}. The value of 1·1 obtained in the test where DCA was added at each dilution was not significantly different from the other values. Thus, the reduction in titre by DCA treatment was small and in no way comparable to the total inactivation of the known susceptible control virus.

TABLE X. ACTION OF DCA ON AFRICAN HORSE-SICKNESS VIRUS

	Reciprocal log LD_{50} titre		Log LD_{50} difference
	DCA-treated	Control	
1. AHS virus	4·0	5·6	1·6
	3·6*	4·7	1·1
	3·7	4·5	0·8
†Kyasanur forest disease virus	<0·5	7·5	>7·0
2. AHS virus	5·8	6·6	0·8
†Langat virus TP–21	<1·8	8·1	>6·3

*DCA added to each ten-fold dilution of virus before incubation.
†Known to be inactivated by DCA.

DISCUSSION

The observations made in this study show that the equine epizootic of 1960 in India was caused by AHS virus, probably of a single antigenic type. Tests carried out at Onderstepoort and at Poona further indicated that this virus was related to Onderstepoort type 6 and indistinguishable from the virus isolated by South African workers from Pakistan. Thus far, all viruses isolated in different countries of West, Middle and South-East Asia belong to this single antigenic type (Director of Veterinary Services, Onderstepoort, 1960; personal communication).

In comparison with serum, whole blood was a much more suitable source for virus isolation. Collection of blood in heparin enabled testing of undiluted blood and was an advantage over the use of the anti-coagulant OCG (potassium oxalate, carbolic acid, glycerine), recommended by the Onderstepoort Laboratory which, because of its toxicity to mice, must be diluted five-fold. Inoculation of infant mice by IC route was the method of choice for virus isolations. Identification of a virus isolate in quick complement fixation test and/or examination of sera for CF antibodies were the simplest methods for establishing diagnosis.

Length and height of viremia are crucial points in the epidemiology of an arthropod-borne virus infection like AHS, as they determine the potential of an infected animal as source for further spread of virus. The only pertinent information in the literature on this subject is an observation of Arnold Theiler (1930) that blood of a horse may be infectious for as long a period as 90 days after onset of disease. This observation was based apparently on a single instance, where disease was believed to be transmitted to a healthy horse by transfusion of blood of a convalescing animal. Subsequent unpublished work in South Africa has not confirmed this long period of viremia (Director of Veterinary Services, Onderstepoort, 1960; personal communication). In our study viremia was detected only up to nine days after onset and it is reasonable to assume that the animal is not infectious thereafter. No information was obtained on the duration and height of viremia before the onset of symptoms.

It was not possible to make an accurate estimate of the mortality rate in the cavalry unit as 48 of the 107 affected animals were sacrificed. If the sacrificed animals were disregarded the mortality would be 36 out of 59, or 61 per cent. However, 24 out of the 48 animals were sacrificed during convalescence (16 or more days after onset) and these may be regarded as 'recovered horses,' as in the naturally fatal 36 cases the average duration of illness was 4·5 days and none lived longer than ten days. Further, if the mortality rate of 61 per cent (the rate in animals not sacrificed) was assigned to the group of 24 horses sacrificed during the first ten days of illness, it would mean that only 15 horses (61 per cent of 24 animals) of the 48 would have died naturally, and the final outcome would have been 51 deaths and 56 recoveries in the 107 affected horses, or a mortality of a little less than 50 per cent. This rate in the cavalry unit contrasts sharply with the mortality rates between 90 to 95 per cent reported for the Rajasthan State and India as a whole (Sahai, 1961) and also other countries affected with the same antigenic type of virus. The difference probably reflects a combination of factors, such as non-recognition of milder cases in the field, reluctance of horse owners to declare the illness in view of the policy in many places of destruction of all sick

animals, greater facility for treatment in the unit, and a better state of health and nutrition of the cavalry horses at the time of onset of illness.

Veterinary authorities in many parts of the world including several states of India enforce immediate destruction of all animals suspected to be suffering from AHS, as this is believed to limit the spread of disease. The rationale for this practice, though not clearly stated, is probably the high mortality rate of 90 per cent and the long period of infectiousness of 90 days associated with the disease. Both of these suppositions are probably not valid. It would seem that destruction of animals after they have already been ill for ten days would serve no useful purpose as they would not be infectious at that time and would have an excellent chance of recovery. Again, is some situations, it may be possible to provide adequate quarantine for the sick animals for the first ten days and to further reduce their chances of *Culicoides* exposure by application of insecticides to the sick animals and spraying in the area. In such situations the usefulness of the policy of destruction needs to be reassessed.

In South Africa, Arnold Theiler and others have hypothesized a 'non-equine reservoir' of AHS virus on epidemiologic grounds, but have not been successful in the actual identification of the 'reservoir' species. Our limited search for antibodies in the animals in the AHS epizootic areas has not given any lead on the species that might be such a 'reservoir'.

The finding of high titres of CF and neutralizing antibodies in 'acute' sera, often with demonstration of circulating virus in the whole blood at the same time, is very curious and may explain the relative inefficiency of serum as source for isolation. Again, the barely detectable action of DCA on this virus is very different from the effect of DCA on many other arbor viruses.

SUMMARY

Strains of virus were isolated from blood or organs of affected horses and were identified as AHS virus. The strains from Rajasthan were antigenically similar to one another and to those from Maharashtra and Uttar Pradesh. The Indian virus was indistinguishable from the virus isolated in Pakistan. The Indian strains were antigenically related to, but different from, South African type 6 virus.

Intracerebral inoculation of infant mice with whole blood was the method of choice for virus isolation as well as for rapid intracerebral passage.

Adaptation to infant mouse brain altered the pathogenicity of the virus for adult mice.

Circulating virus was demonstrable up to nine days after onset in titres ranging from 'traces' to $10^{-1 \cdot 8}$ except for one specimen where the titre was $10^{-4 \cdot 6}$.

DCA inactivated the virus to a very small extent.

The mortality rate in the Jaipur cavalry unit was estimated at a little less than 50 per cent. The ratio of clinical to sub-clinical infection was estimated as 15:1.

Sera of some dogs and donkeys in the epizootic areas neutralized AHS virus. Such activity was not detected in sera of man, goats, cattle and birds.

ACKNOWLEDGEMENTS

Much of the above work was based on the study of the epizootic at Jaipur where the Commandant and Veterinary Officers of the unit rendered invaluable help. The strains from West Khandesh, Maharashtra, and U.P. were isolated from specimens submitted by Dr. D. S. Gorhe (Veterinary College, Bombay) and Dr. D. K. Murthy (Livestock Research Station, Mathura) respectively. The donkey sera from Jaipur were obtained by Dr. Daulat Singh and Dr. Budh Singh of the Department of Animal Husbandry, Rajasthan State. The survey sera listed under the heading ' Maharashtra (2) ' in Table VII were collected by Drs. Sapre, Phadke and Bhagwat of the Department of Animal Husbandry, Maharashtra State.

Complement-fixation tests were carried out by Dr. Tara B. Gokhale. Excellent assistance was given by Miss Farida G. Maniar in the organization of data.

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PRELIMINARY REPORT ON CLINICAL SYNDROMES AND PATHOLOGICAL LESIONS IN MILK TETANY IN CALVES

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DUNCAN *et al.* (1935) reported that failure to raise calves on a ration of supplemented milk was associated with low plasma magnesium. The calves exhibited tetany and other symptoms indistinguishable from those associated with low serum calcium. Huffman and Duncan (1936) reported that the symptoms and lesions in calves on milk diet could be prevented by adding magnesium compounds to the diet. Moore *et al.* (1938) reported the presence of calcified plaques on the endocardium, wall of aorta and general calcification of soft tissues in 'milk syndrome'. Nephritis and marked hyaline degeneration of skeletal musculature with concomitant calcification were also observed.

Blaxter *et al.* (1954) produced magnesium deficiency in calves by feeding them on artificial diet. They reported that uncomplicated magnesium deficiency in calves aged 50–120 days did not esult in the pathological calcification of the endocardium and soft tissues as reported in milk syndrome. They were of the opinion that the lesions observed by Moore *et al.* (1936, 1938) in milk syndrome appeared to be almost identical with those observed in experimental vitamin E deficiency, in the naturally occurring muscular dystrophy and in cod-liver oil poisoning (Blaxter *et al.*, 1953). Since in the experiment of Duncan *et al.* (1935), which provided the material for Moore *et al.* (1938), cod-liver oil was included in the ration, it is possible that dystrophic degeneration due to cod-liver oil poisoning complicated the interpretation of pathological findings (Blaxter *et al.*, 1954).

The present paper reports calcification of aorta, skeletal muscle and other soft tissues of calves kept exclusively on milk diet with no supplementation of minerals and cod-liver oil.

MATERIAL AND METHODS

Four calves, three of them from birth (No. 300, 301 and 302) and another (No. 294) about two-month-old, were fed exclusively milk at the rate of 0·5 kg. of milk per 4·5 kg. body-weight. The calves were muzzled all the time so that they could not graze or take anything other than milk. They were weighed and bled every fortnight for estimation of serum magnesium, calcium and inorganic phosphorus. A similar number of calves of the same age group and maintained on their normal ration, i.e., milk, concentrates and roughage according to their age, were kept as control. The methods employed in estimating the above minerals have been reported by Ray and Mullick (1963). Tissues obtained from the dead or sacrificed animals were fixed in

10 per cent formol saline and after necessary processing were embedded in paraffin and 5-6 μ thick sections were cut and stained by haematoxylin and eosin.

RESULTS

Clinical symptoms: The experimental calves began to exhibit symptoms of hyperexcitability when their initial serum magnesium levels of 2·5 mg./100 ml. were lowered to 1·5 mg./100 ml. and below. It took about 35-50 days of milk feeding to bring them down to such levels of magnesium in serum. The symptoms of hyperexcitability and irritability were exaggerated when the serum magnesium levels had been further lowered to 1·1 mg./100 ml. and below. The calves were hypersensitive. The superficial reflexes were greatly exaggerated. The experimental calves were constantly lifting their heads upwards with stretched necks as if trying to catch some imaginary object. Slightest sound from outside the stall would throw the animal into agitation accompanied with muscular tremors of different groups of muscles.

Tetany was observed for the first time in calf No. 301 on the 57th day of feeding of milk from birth. When let out from the stall it ran a few yards, fell down and developed clonic tetany of muscles all over the body. The limbs were rigid with joints flexed. The calf got up after about two minutes and then returned to normal. The calf was quite normal afterwards. The serum magnesium level of this calf, two days before the first attack of tetany, was 0·96 mg./100 ml. The calf at the age of seven months and ten days developed all of a sudden weakness and incoordination of gait of the forelimbs, and fell down prostrate with loss of sensation in the legs. It lay prostrate and was sacrificed on the third day. The body emitted characteristic smell of urea a day prior to slaughter.

Calf No. 302 developed convulsions for the first time after 51 days of milk feeding. It succumbed to the first attack of tetany and convulsion. The serum magnesium level, a day prior to the fatal attack, was 0·98 mg./100 ml. Calf No. 294, which was about two-month-old when included in the experimental group, had the first attack of tetany at the age of six months and 27 days. It survived the attack and was slaughtered on the following day in order to see morbid anatomy, if any. Serum magnesium level on the day of sacrifice was 1·02 mg./100 ml. Calf No. 300, which was on milk diet from birth, surprisingly had no attack of tetany even though it touched a low serum magnesium level of 1·2 mg./100 ml.

Post-mortem observations: CALF No. 302: It died on the first attack of convulsion and tetany after 51 days of milk feeding. The carcass was fair in condition with plenty of fat around the kidneys, heart, subcutaneous tissue and mesentery. Lungs showed many hard, pea-sized nodules with a bluish tinge along the interlobular septa and at other places. On sectioning, these nodules were found to be gritty masses of greyish-blue substance. Aorta did not show any evidence of calcification on naked-eye examination. Liver showed many suppurative foci with inspissated pus. Such foci were quite hard and gritty.

Two other experimental calves (Nos. 301 and 294), which were sacrificed, did not show any foci of calcification macroscopically. Kidneys of calf No. 301, which lay prostrate for three days prior to slaughter and emitted urea odour, were pale in

colour; the cortex showed grey opaque spots. Urinary bladder was distended with dark-coloured urine.

Histopathological studies: Histopathological changes observed in the tissues of calves Nos. 302 and 301 are described below.

AORTA: The most conspicuous pathological change in the aorta of calf No. 302 was calcification of elastic fibres of intima. Such calcified elastic fibres by haematoxylin and eosin staining looked slightly bluish-red, as the calcified fibres had taken both the stains. Those elastic fibres which had not been calcified but had undergone hyaline degeneration looked more reddish. By von Kossa staining the calcified fibres looked black (Fig. 1). Over a considerable length of the intima the elastic fibres had been calcified and at other places were degenerated and hyalinised. The calcified fibres varied in thickness. At some places calcification was confined to the thin layer of fibres, whereas at other places thick plaques of calcification were distinct. The muscle fibres in the deeper intima were loosened up and degenerated, but here the calcification was not conspicuous. The elastic and muscle fibres could be differentiated by the loss of wavy character in the former in Verhoeff's elastic staining.

Aorta of calf No. 301, which was sacrificed at the age of seven months and 14 days, did not show diffused areas of calcification of elastic fibres as were seen in calf No. 302. Only scattered areas of calcification of elastic fibres of intima and media were seen. Such areas of calcification were confined to a few strands of elastic fibres.

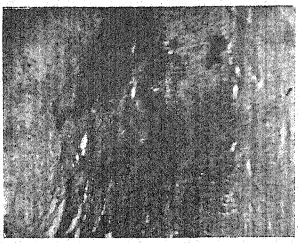
KIDNEYS: Kidneys of calf No. 302 showed extensive areas of calcification of degenerated cells in the cortex and medulla (Fig. 2). Such areas of calcification looked bluish, as they had taken up deep haematoxylin stain which is suggestive of calcification. This was confirmed by von Kossa's method of staining for calcium. Glomeruli showed increased proliferation of endothelial cells, and red blood cells and granular debris were seen in the space under the Bowmann's capsule of some of the glomeruli.

The afferent arterioles, arcuate artery and intertubular arteries showed hyperplastic sclerosis. There was complete obliteration of lumen of some of the arterioles as a result of proliferation and thickening of intima.

Kidneys of calf No. 301 showed marked degenerative changes of the tubules. Some of the tubules were greatly dilated and contained homogeneous hyaline casts of albuminous material, desquamated epithelial cells and polymorphonuclear leucocytes. Mononuclear cells had infiltrated around the sclerosed blood vessels and degenerated tubules at places (Fig. 3).

LUNGS: Lungs of calf No. 302 showed extensive oedema, inflammation of pleura and suppurative areas with calcification of pus, which was found to be gritty on macroscopic examination. The alveoli and interstitial tissue showed extensive oedema by the presence of homogeneous deeply acidophilic fluid in the alveoli and in the interstitial tissue.

The suppurative areas consisted mainly of serous fluid and polymorphonuclear leucocytes, most of which showed karyolysis, pyknosis or karyorrhexis of their nuclei. Pus in these areas had been calcified, as indicated by deep haematoxylin staining and by von Kossa's method for calcium. The sudden death of the animal could be explained on the basis of extensive oedema of lungs which brought about failure in circulation and heart failure.



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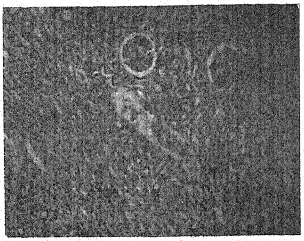

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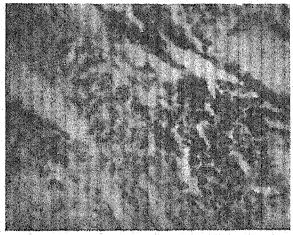

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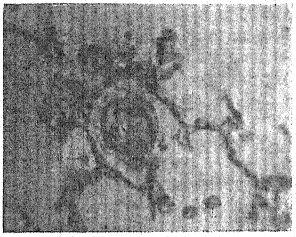

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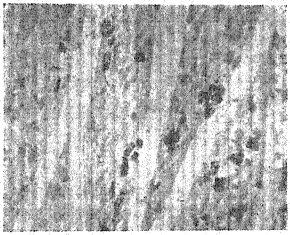

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FIG. 1. SECTION OF AORTA SHOWING CALCIFICATION OF ELASTIC FIBRES OF INTIMA. VON KOSSA'S STAIN × 63.
FIG. 2. SECTION OF KIDNEY OF CALF NO. 302 SHOWING SCATTERED AREAS OF CALCIFICATION IN THE CORTEX AND MEDULLA. HAEMATOXYLIN AND EOSIN × 63.
FIG. 3. SECTION OF KIDNEY OF CALF NO. 301 SHOWING MONONUCLEAR INFILTRATION AROUND THE SCLEROSED BLOOD VESSELS. HAEMATOXYLIN AND EOSIN × 275.
FIG. 4. SECTION OF LUNG OF CALF NO. 301 SHOWING FIBROUS THICKENING, HYALINISATION AND OBLITERATION OF THE LUMEN OF AN ARTERIOLE. HAEMATOXYLIN AND EOSIN × 275.
FIG. 5. SECTION OF SKELETAL MUSCLE OF CALF NO. 301 SHOWING CALCIFICATION OF MUSCLE FIBRES. VON KOSSA'S STAIN × 63.

Lungs of calf No. 301 also showed oedema of alveoli and interstitial tissue. The small arterioles of the lungs showed fibrous thickening of the intima. The fibrous tissue and the replacing media had undergone hyalinisation and the vessels looked like hyaline cylinders with greatly obliterated lumen (Fig. 4).

SKELETAL MUSCLE: Skeletal muscle of calf No. 301 showed hyaline degeneration of groups of muscle fibres with loss of nuclei and striation. Such fibres looked intensely acidophilic. Some of the groups of muscle fibres after degenerative changes were feebly stained and vacuolated from autolysis; some of these had assumed slightly basophilic colour with haematoxylin and eosin staining, which was suggestive of calcification. When stained with von Kossa's method for calcium these areas were stained black (Fig. 5).

The skeletal muscle of calf No. 302, which had died after first attack of convulsion, did not show calcification of muscle fibres.

LIVER: Liver of calf No. 302 showed suppurative foci. The suppurative areas consisted mostly of serum and degenerated neutrophils. Grittiness of liver parenchyma noted at post-mortem was due to inspissated pus. The portal tracts were very much infiltrated with lymphocytes and fibroblasts.

DISCUSSION

Blaxter *et al.* (1954) did not find pathological calcification of the tissues or formation of calcified plaques in the aorta and endocardium of calves, which had exhibited tetany and had been maintained on magnesium-deficient diet for long periods. They were of the opinion that hyaline degeneration of the muscles observed by Moore *et al.* (1938) was not due to the deficiency of magnesium or interference with magnesium metabolism, but perhaps due to vitamin E deficiency induced by cod-liver oil in the milk ration. In the present experiment calves were fed exclusively on milk diet with no supplement of cod-liver oil or either minerals; yet pathological calcification of aorta and other soft tissues were observed. There was unmistakable evidence of calcification of elastic fibres of aorta of calf 302 following degenerative changes. The calcification noted in the lungs, however, of the same animal was obviously due to secondary infection by pyogenic organisms. This resulted in suppurative foci in lungs and liver, and calcification of the inspissated pus in the former which could take place irrespective of magnesium deficiency in the diet or hypomagnesaemia. However, the calcification of aorta of calf No. 302 and of skeletal muscle of calf No. 301 seem to be associated with dietary factors. The calcification of skeletal muscle of calf 301 could be ascribed to vitamin E deficiency because milk contains very little of a-tocopherol. Even after conceding that calcification of skeletal muscle could perhaps be due to vitamin E deficiency the calcification noted in the aorta of calf 302 seems to be related to hypomagnesaemia. The authors are not aware of any reference purporting to establish that vitamin E deficiency can lead to cal ification of aorta and kidneys. The massive haemorrhages and venous necrosis in the heart and vascular system in magnesium-deficient calves as reported by Blaxter *et al.* (1954) were not seen in any of the autopsied calves.

The symptoms of tetany were observed after about eight weeks of milk feeding. During the first four weeks only mild hypomagnesaemia was observed. Parr (1957) also reported that clinical symptoms of tetany became apparent in calves fed on rations of whole milk after eight weeks. Symptoms of tetany and convulsions were observed in the experimental calves when the Ca:Mg ratio was 8 or 10:1 whereas in normal blood it is 4 or 5:1. Duncan *et al.* (1935) also reported that the calves fed on a ration of milk or milk with supplements developed tetany when the Ca:Mg ratio was 8 or 10:1. Growth was quite normal during the first few weeks of milk feeding, but was inhibited later on as the experiment progressed. Maynard *et al.* (1956) reported that lowering the magnesium content of the diet in guinea-pigs by 70 per cent resulted in poor growth, enlarged and damaged kidneys, a very large increase in the calcium content of the kidneys and a smaller increase in liver. When the calcium and phosphorus content of the diet was lowered, these deleterious effects did not occur.

MacIntyre and Davidson (1958) reported nephrocalcinosis in rats and guinea-pigs fed magnesium-deficient diet. They reported progressive loss of magnesium content of skeletal muscle to 70 per cent of the control values. The calcium content of muscle, brain and liver did not differ significantly. They also reported that nephrocalcinosis occurred only when hypercalcaemia was also present. Magnesium is important in the transport of calcium in the renal tubules and even short periods of hypomagnesaemia may be deleterious (MacIntyre, 1959). According to MacIntyre nephrocalcinosis was produced in magnesium-deficient rat and it was accompanied by hypercalcaemia. In our experimental calves the levels of calcium in blood were quite normal, yet calcification was noticed in the aorta, kidneys and skeletal muscle. It seems that the disturbed calcium magnesium blood ratio aids calcification of degenerated tissues at times.

The present investigation, though limited to a small number of calves, points to the possibility that hypomagnesaemia in calves resulting from feeding milk alone for more than two months results in calcification of vascular system and soft tissues.

SUMMARY

Calves developed symptoms of tetany and convulsions after about eight weeks of milk-feeding, starting from birth. Clinical symptoms and histopathological changes are described in detail. Histopathological studies revealed calcification of aorta, skeletal muscle, lungs and kidneys in some of the experimental calves.

ACKNOWLEDGEMENTS

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STUDIES ON THE SEROLOGY OF *STREPTOCOCCUS AGALACTIAE* – SIMPLIFIED SLIDE DOUBLE DIFFUSION AGAR PRECIPITIN TECHNIQUE*

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THE principle of analysing antigen by diffusion in gel was demonstrated as early as 1905 by Bechhold, who showed that diffusion of an antigen into gel incorporated with the homologous anti-serum may bring the appearance of one or more lines of precipitin. Reiner and Kopp (1927) obtained parallel results by allowing pig serum to diffuse in an agar containing rabbit anti-pig serum and observed five or six precipitin lines. Nicolle *et al.* (1920) indicated modification of this technique for assaying diphtheria and tetanus toxins and their corresponding anti-toxins.

Oudin (1946, 1947, 1948a, b, 1949), who made a detailed study on investigation of immuno-technique, suggested that each precipitin line represents the specific antigenic component of an antigen allowed to diffuse against the homologous antibodies in an agar gel. He described two methods by means of which antigens responsible could be identified. These methods could be used to determine the minimum numbers of antigenic components present in a mixture and applied it to demonstrate the antigenic complexity of horse serum and colostrum. Munz and Becker (1950) confirmed Oudin's method by demonstrating the antigenic pattern of horse serum albumin, bovine serum albumin and egg albumin. Becker *et al.* (1951) worked on a theoretical discussion of the factors affecting the results of immuno-diffusion in gel.

Diffusion technique in plates, as described by Ouchterlony (1948, 1949, 1953) and by Elek (1948, 1949a, b, c, d), seems especially suitable for bacterial antigen and antibody systems in high concentration. Ouchterlony employed this technique to study the toxicity and strains of *C. diphtheriae*; he was the first to demonstrate the antigenic relationship amongst different strains within the genus. Elek (1949) also demonstrated the possibility to identify the antigens responsible for some of the lines by observing their indicating effect. The method was further explored by Elek and Levy (1950) while studying the system of toxigenic staphylococci. From Ouchterlony's (1948, 1949, 1953) studies and the experiments on specific inhibition of precipitin lines performed by Bjorklund (1954) it can be assumed that every single line has to be considered a specific reaction between one particular antigenic component and its corresponding antibody. Wilson and Pringle (1954) demonstrated that the temperature of incubation had a pronounced effect on the rate of development but not in the final

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appearance of precipitin lines. They also worked to find out suitable spatial pattern for the reservoirs in the diffusion plate, but could not find out ideal arrangement. Ouchterlony's modification of the plate immuno-diffusion precipitin technique appears to represent a valuable tool for the qualitative analysis of immunogenic system, especially when comparative studies of antigens or antibodies are desired.

Numerous modifications to the immuno-diffusion agar precipitin technique developed by Ouchterlony have been suggested by different workers in the recent years. Wadsworth (1957) described a micro-double diffusion agar precipitin test. Crowle (1958) described not only the procedure for carrying out the micro-double diffusion agar precipitin test but also the methods of washing, staining and preserving the precipitin reaction. Mansi (1958) employed the slide gel diffusion drecipitin test with different viral antigen-antibody systems and put forward the advantages of this technique. The present study was carried out with a view to explore the possibility of a simplified micro-slide test for the *Streptococcus agalactiae* system.

MATERIAL AND METHODS

Preparation of antigens: The acid extract, Brown's modification (1938) of Lancefield's method (1933) and autoclaved extract (Thulin, 1948), were prepared from different strains of Group B streptococci isolated from clinical cases of bovine mastitis in India. Some other strains representative of Lancefield types were also included to have a comparative study.

One hundred millilitres of an 18-20-hour glucose broth culture was centrifuged after testing for purity of the culture. The deposit was washed twice with isotonic phosphate-buffered saline (pH 7·2) in 15 ml. graduated centrifuge tubes. The supernatant was removed to leave the deposit as dry as possible. A 1:25 dilution of the packed cells was made in buffered saline and the extracts were prepared. The supernatant, which formed the extract, was ampouled and stored in the refrigerator for day-to-day use.

Preparation of antisera: Four Group B strains were used for immunizing rabbits to prepare antisera against live whole cultures. The four strains were grown in glucose broth at 37°C for 18-20 hours, centrifuged for 30 minutes at 2,000 r.p.m. and were washed twice with physiological saline. The washed cells were suspended in physiological saline to match opacity tube No. 6 of the scale of Burrows Wellcome and Co., and used as antigen for injecting rabbits after testing the purity. The antigens were stored in the refrigerator for day-to-day use. After pre-immunization bleeding, rabbits were inoculated intravenously with an initial dose of 0·25 ml. for five consecutive days and five days later with 0·5 ml. for five consecutive days. Subsequent courses of five days each with 1 ml. dose were administered allowing a rest period of five days in between the two courses of injections, until serum of suitable titre was obtained. The antisera were labelled as per the number of the rabbits immuni ed.

Medium for slide double gel-diffusion test: This was prepared by dissolving in glass-distilled water-purified agar powder (Davis, New Zealand) to a final concentration of 1·5 per cent with sodium chloride (0·85 per cent), and merthiolate (1:10,000) added to it.

Preparation of slides: New microscopic slides free from scratches and cloudiness were kept immersed in methyl alcohol. For use they were rinsed in distilled water, air-dried and stored in a dust-free container. Two thin strips of zinc oxide adhesive tape were fixed on the slides in such a manner as to provide a working area of about 4-5 cm. with about 2 cm. free space at each end of the slide for manipulation. The strips of tape helped to limit the spread of the medium and also to provide a fairly constant depth on the slide. About 1-2 ml. of melted agar was distributed over the slide between the two strips of zinc oxide tape by means of a Pasteur pipette so as to give an even layer. The slides were allowed to cool till the medium solidified and they were then stored in a refrigerator kept in petri-dishes for day-to-day use. The pattern of wells or reservoirs for the reagents to be drilled into the agar on the slides was first drawn on a graph paper. Different arrangements of reservoirs were used for various purposes. The pattern of the wells was so arranged that four of them were around and equidistant from one in the centre. Diameter of each well was 6 mm. and the distance from the centre of the middle well to that of the peripheral one was 10 mm., which was found to be the best for getting the optimum results. The slide with agar bed was kept over the graph; reservoirs were cut into agar at the appropriate place with sterile metal case of a clinical thermometer, in which a hole was drilled at the closed end and fixed to 10 ml. rubber bulb. The sharp end of the thermometer case was made to cut through the agar bed over the reservoir pattern drawn on the graph paper. The reservoirs were made by suction with the help of the rubber bulb.

Slide double diffusion agar precipitin technique: A single slide was used for testing six antigens by arranging reservoirs in a pattern as illustrated in various figures. Central rows of the well contained antisera, while rows towards the margin of the slide contained the antigens to be studied. Drying was prevented by keeping these slides in petri-dishes and placing petri-dishes one above the other on a stand in a glass chamber. The lid of the glass chamber was tightly fixed to minimize evaporation at the desired temperature of 20° C. Readings were made at room temperature as quickly as possible. Visual inspection could be performed conveniently by holding the slide horizontally over a microscope lamp provided with a blue filter and slanted upward at an angle of about 45°. All the slides were photographed when the precipitin lines approached maximum development and also at the termination of the experiments. The photographs were taken according to standardized technique by placing the uncovered slide directly on Kodak contrast lantern plate and exposing it to the transmitted light of a fluorescent illuminator.

Staining of double diffusion agar slides: The slides were kept in phosphate buffered saline, pH 7·2, overnight and later were transferred for two minutes to a bath containing glass-distilled water. Slides thus washed were placed in a staining bath containing 0·1 per cent solution of azocarmine in 1 per cent acetic acid for two to three minutes. They were then kept in 1 per cent acetic acid for two minutes before transferring to another bath containing 1 per cent acetic acid and 1 per cent glycerol in which they were kept for one minute. The slides were finally dried at 20° C. before storing. Care was taken to avoid mechanical agitation or stirring during the staining process.

RESULTS AND DISCUSSION

To enhance the contrast between the agar surface and the precipitin lines efforts were made to stain the slides using azocarmine dye. Although preliminary investigations indicated the usefulness of the method, detailed study using different staining methods will be needed to find the staining method of choice. A few of the preparations of slide double gel-diffusion tests are presented in Figs. 1–3. Azocarmine was employed for staining purposes as, being an acid dye, it reacted with polysaccharide precipitin reactions which are encountered in this particular system; non-specific reaction due to other factors will not be brought out when this type of staining reaction is used. After staining the reaction lines were seen more clearly and they were also better defined. The stained preparations could be stored without deterioration for at least one year or even permanently.

SCHEMATIC DIAGRAMS

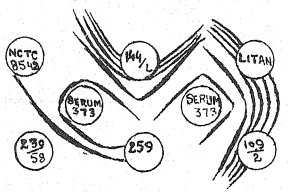

FIG. 1. AZOCARMINE G-STAINED PRECIPITIN LINES FORMED BETWEEN *Str. agalactiae* ANTISERUM 373 AGAINST AUTOCLAVED EXTRACTS NCTC 8542, 144/L. LITAN, 259, 230/58

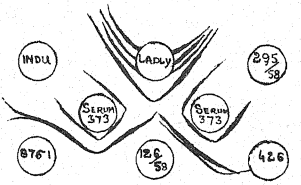

FIG. 2. AZOCARMINE G-STAINED PRECIPITIN LINES FORMED BETWEEN *Str. agalactiae* ANTISERUM 373 AGAINST AUTOCLAVED EXTRACTS INDU, LADLY 295/58, 426, 126/59 & B76. 1

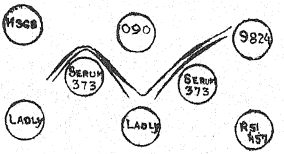

FIG. 3. AZOCARMINE G-STAINED PRECIPITIN LINES FORMED BETWEEN *Str. agalactiae* ANTISERUM 373 AGAINST AUTOCLAVED EXTRACTS 090, H36B, 9824, R51, 457 & LADLY

Numerous modifications to Ouchterlony double diffusion agar precipitin technique have been developed by using microscopic slides (Wadsworth, 1957; Crowle, 1958; Mansi, 1958). In the present study to explore the possibility of a slide test for *Str. agalactiae* system and to investigate the merits and demerits of this method, better results than those obtained with serological and other tests were obtained.

Besides, the test could be performed with very little quantity of reactants and the results could be read in much shorter time. The relative sensitivity of the slide test may perhaps be due to the fact that the antigen and the antibody components can diffuse much better in a smaller volume and thinner layer of agar giving quicker and more sharp reaction.

The study proved the utility of this technique and opens the field for adopting it as a routine method for serotyping of *Str. agalactiae* strains isolated from cases of mastitis. It would appear that the gel diffusion test might replace other conventional methods, namely, precipitation, agglutination, etc., for serotyping of streptococci of mastitis origin and employed for diagnostic work in the field for various bacterial and viral diseases.

SUMMARY

The simplified double diffusion agar precipitin technique using *Str. agalactiae* system is presented as a modified, complete procedure for frequent use for diagnostic work in the field for various bacterial and viral diseases. The procedure has proved more convenient, more sensitive and economical in all respects than other conventional agglutination or precipitation tests emlpoyed in serotyping streptococci.

Details are given for putting up the test, staining and preserving the precipitin reactions.

ACKNOWLEDGEMENTS

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ON EIMERIAN OOCYSTS RECOVERED FROM INDIAN CAMEL (*CAMELUS DROMEDARIUS*)*

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COCCIDIAN parasites of camel have received very little attention in India. Henry and Masson (1932a, b), in case of dromedary (*Camelus dromedarius*) in France, described large oocysts as measuring 80-100$\mu \times$65-80μ in size with a thick wall ranging from 10·5-15·6μ in thickness, having a micropyle at one pole and measuring 10-14μ in diameter. For this he suggested the name *Globidium cameli* resembling horse coccidium, *G. leukarti*. The macrogametocytes, schizonts and microgametocytes were also described. Nöller (1932) in Germany, from an examination of 20 camels (*Camelus bactrianus*) autopsied, found coccidian oocysts in eight, of which three were heavily parasitised. The oocysts were described as round to elongate in shape, measuring 32$\mu \times$25-27μ in size with a micropyle of 5-7μ in diameter and having a cap at one end. This material was assigned to a new species, *Eimeria cameli*. Its inadequate description without any illustrations resulted in a confusion which still continues. Subsequently, Enigk (1934) described, for the first time from the small intestine of three camels, the schizonts and microgametocytes of *E. cameli* earlier described from Germany. The endogenous stages of *G. cameli* in small and large intestine were also met with.

Unaware of Nöller's work, Iwanoff-Gobzem (1934) discovered in 1932 two types of coccidian oocysts, oval and round, and noticed polar granule and sporocystic residuum in most of them. These were also assigned to a new species, *Eimeria cameli*. Yakimoff (1935), who also observed the types of oocysts earlier reported by Iwanoff-Gobzem, described the presence of a 'cap' in some of the oval forms. Yakimoff and Matschoulsky (1939), after repeated faecal examinations of camels kept at Leningrad Zoological Gardens, detected both the oval and round oocysts and, for the first time, differentiated these on the characters of shape, presence or absence of a polar cap and of sporocystic residuum. Thus, the confusion in regard to *E. cameli* was to a great extent cleared up, as *E. cameli* was retained for the round oocysts which lacked a cap but had a sporocystic residuum, while the oval forms which were capped but had no sporocystic residuum were assigned to a new species, *E. dromedarii*. Nöller's material, according to them, belonged to *E. cameli*. Hardcastle (1943) listed the two species—*E. cameli* Nöller, 1932, emend. Yakimoff and Matschoulsky, 1939 with *E. cameli* Iwanoff-Gobzem, 1934 proparte as its synonym and *E. dromedarii* Yakimoff and Matschoulsky, 1939. Evidently, Henry and Masson's material of 1932 was not considered by these authors.

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Tzygankov (1950), in a contribution on the revision of coccidian fauna of camels in U.S.S.R., synonymised *E. dromedarii* with *E. cameli* and added to the list of recognized species the new form, *E. kazachstanica*. He (1955) also reported the occurrence of *E. cameli*, *E. kazachstanica* and *Isospora orlovi* Tzygankov, 1950 in camels. Reichenow (1953), considering the nature of gamogonic and sporogonic stages, transferred *G. cameli* to the genus *Eimeria* for inclusion under the subgenus *Globidium* and listed it as *Eimeria* (*Globidium*) *cameli* Henry and Masson, 1932 (Pols, 1960). Pellérdy (1956) in his valuable catalogue of the genus *Eimeria* Schneider, 1875 made, for the first time, a critical analysis of the various species and transferred *G. cameli* of Henry and Masson, 1932 and *Eimeria* (*Globidium*) *cameli* of Reichenow (1953) to the genus *Eimeria*. As the name *E. cameli* was preoccupied for Nöller's material, *E. nölleri* was proposed for this parasite and *E. kazachstanica* was synonymised with *E. nölleri*. The three species considered valid were: *E. cameli*, *E. dromedarii* and *E. nölleri*.

Abdussalam and Rauf (1957), evidently following Reichenow (1953), identified the oocysts from one-humped camel in Lahore as belonging to *Eimeria* (*Globidium*) *cameli* (Henry and Masson, 1932), *E. nölleri* Reichenow, 1953 and *E. dromedarii*, Yakimoff and Matschoulsky, 1939. Prasad (1960) described *E. pellerdyi* from *Camelus bactrianus* kept at London Zoological Gardens, differentiating his new form from *E. nölleri* and *E. dromedarii* but not from *E. cameli*. He appears to have regarded only two eimerian species, *E. dromedarii* and *E. nölleri*, as occurring in camel. He observed that Nöller had redescribed this species (*G. cameli*) which was earlier described by Henry and Masson, and also mentioned that Nöller had, in general, agreed to the description given by Henry and Masson. This observation seems erroneous as Henry and Masson's work does not appear in Nöller's paper, because *Eimeria* and *Globidium* were at that time treated as separate genera. Prasad's observation that Yakimoff and Matschoulsky considered the oval forms to be *E. cameli* and round forms as *E. dromedarii* also appears erroneous. Recently, Dubey and Pande (1963) described, in a preliminary report, a new species *E. rajasthani* from *Camelus dromedarius*.

According to Pellérdy (1963) the genus *Eimeria* is represented by (1) *E. cameli* Nöller, 1932, emend. Yakimoff and Matschoulsky, 1939. Synonyms: *E. cameli* Iwanoff-Gobzem, 1934 not *Globidium cameli* Henry and Masson, 1932, not *Eimeria* (*Globidium*) *cameli* Reichenow, 1953; (2) *E. dromedarii* Yakimoff and Matschoulsky, 1939. Synonyms: *E. cameli* Nöller, 1932, proparte, *E. cameli* Iwanoff-Gobzem, 1934, proparte; (3) *E. nölleri* (Reichenow, 1953) Pellérdy, 1956. Synonyms: *Eimeria* (*Globidium*) *cameli* (Henry and Masson, 1932) Reichenow, 1953, *E. kazachstanica* Tzygankov, 1950 not *Eimeria* (?) *nölleri* Rastigaieff, 1930, and (4) *E. pellerdyi* Prasad, 1960. To this list *E. rajasthani* Dubey and Pande, 1963 has to be added.

MATERIAL AND METHODS

Forty-five faecal samples were collected from camel-calves of less than ten months of age at the Camel Breeding Farm, Bikaner (Rajasthan). None of the infected animals showed any clinical signs. The faecal samples were taken directly from the rectum and were immediately placed in 10 per cent formalin and 2·5 per cent potassium dichromate solution. They were allowed to sporulate at room temperature and then examined under Baush and Lomb microscope with achromatic objectives following

concentration by the usual centrifugal sugar flotation technique. Some of the oocysts were stained with eosin-iodine solution (Christensen, 1938). To avoid the optical illusion, few oocysts were ruptured under pressure.

RESULTS AND DISCUSSION

Twenty-eight of the faecal samples contained coccidia which on study belonged to three eimerian species: *E. rajasthani* Dubey and Pande, 1963, *E. dromedarii* Yakimoff and Matschoulsky, 1939 and *E. nölleri* (Reichenow, 1953) Pellerdy, 1956.

E. rajasthani: Subsequent to the submission of the preliminary note on the oocyst of this species, which was differentiated from the forms known from the then available literature, more details regarding some of the described species were obtained through the courtesy of Dr. Pellérdy (personal communication, 1963), who gave the chief features characterising the valid forms with their synonyms. Accordingly, *E. rajasthani* needs detailed comparison with *E. cameli*, *E. dromedarii*, *E. nölleri* and *E. pellérdyi* in view of this newer concept.

E. cameli has round or subspherical oocysts (Fig. 1) which, with a wide micropyle, are without a cap (In *E. rajasthani* the oocysts have nearly ellipsoidal shape, are without a visible micropyle but have a well-defined cap). The sporocysts (Fid. 2) in *E. cameli* are lemon-shaped, while in *E. raj sthani* they are nearly ovoid and have a prominent thickening (stieda body) at the narrower end. *E. dromedarii* has oval or subspherical oocysts which are truncate at one end and have a polar cap; their size being smaller than in *E. rajasthani*. They also differ in the absence of a stieda body and sporocystic residuum. *E. nölleri* is characterised by oocysts (80-110 $\mu \times 65$-60 μ) of much bigger

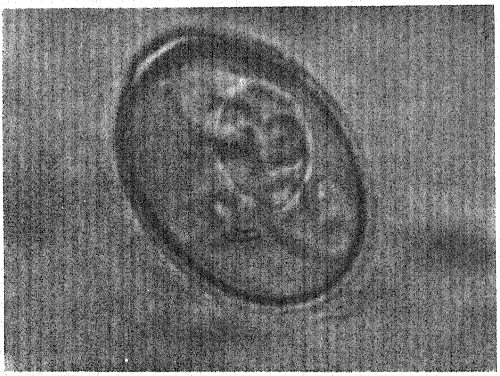

FIG. 1. PHOTOMICROGRAPH OF A SPORULATED OOCYST OF *E. rajasthani* SHOWING THE POLAR CAP, CHARACTER OF ITS WALL, CONTOUR OF A SPOROCYST (IN FOCUS) WITH ITS TWO SPOROZOITES. X 1,600.

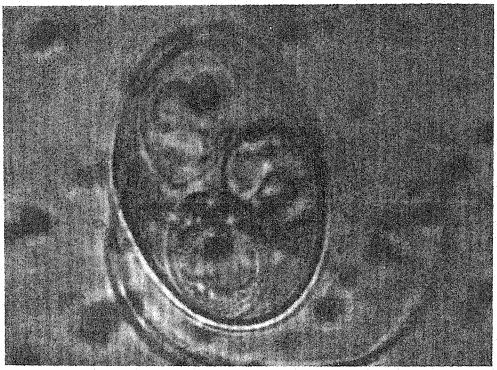

FIG. 2. PHOTOMICROGRAPH OF A RUPTURED OOCYST OF *E. rajasthani* WITH SPOROCYSTS INSIDE ITS INNER WALL EXHIBITING THE THICKENING AT THE NARROWER END (DISTINCTLY VISIBLE IN TWO) AND THE BROKEN OUTER WALL. X 1,800.

size and with a markedly thick three-layered wall. The oocysts in *E. pellérdyi* are smaller, oval or elliptical in shape and without a cap.

E. dromedarii: This species was found in 20 (44 per cent) camels. The oocysts were capped and subspherical to spherical. Hundred oocysts measured 26-28μ × 21-23μ in size with a mode of 27 $\mu \times 21$ μ; their length-width ratio was 1·19:1·33. The oocyst wall was 2-3 μ thick, had two layers, an outer thicker and light yellowish-green layer and an inner darker layer with a shining inner contour. The oocystic cap was 5-7 μ wide and 2-3 μ high. No micropyle was visible. The sporont measured 16-18 μ in diameter (Fig. 3). The sporulation time was not determined, but oocysts had sporulated by the ninth day after collection. Oocyst and sporocyst residue and oocyst polar granule and stieda body were absent. Sporocyst wall was composed of single layer. Fifty ovoid sporocysts measured 10-11 $\mu \times 8 \cdot 5$ μ with a mode of 10 $\mu \times 8.5$ μ. Each sporozoite contained two or more prominent globules (Fig. 4).

The only difference in the account given by Pellérdy (Personal communication, 1963) relates to the thickness of the oocystic wall which, unlike our observations of 2-3 μ thick, has been given as 0·8-1·4 μ thick. Besides, the wall has been described as brown. Further in proposing this species, Yakimoff and Matschoulsky had restricted it to the oval oocysts that had a cap but Pellérdy mentioned the shape as oval or subspheric. This species does not seem to have so far been reported from the Indian camel.

E. nölleri: A single, unsporulated, ovoid oocyst was encountered during faecal examination; it measured 67 $\mu \times 57$ μ in size. The oocystic wall had three layers: the outermost thick, pitted and of greenish-yellow colour, the middle darker, and the innermost dark-green. A micropyle was visible at the anterior end. The centrally-placed

sporont measured 41 μ in diameter (Fig. 5). This oocyst evidently belonged to *E. nölleri*, which does not seem to have yet been reported from Indian camel. Data on its sporulation could not be completed.

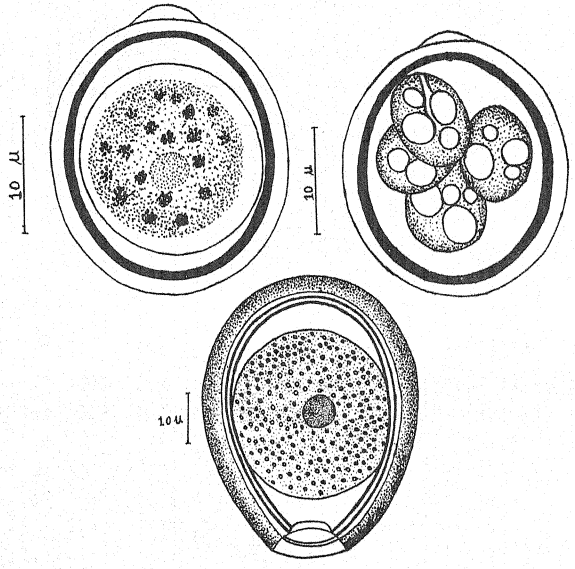

FIG. 3. CAMERA LUCIDA DRAWING OF AN UNSPORULATED OOCYST OF *E. dromedarii*.
FIG. 4. CAMERA LUCIDA DRAWING OF A SPORULATED OOCYST OF *E. dromedarii*.
FIG. 5. CAMERA LUCIDA DRAWING OF AN UNSPORULATED OOCYST OF *E. nölleri*.

The oocystic characteristics of all the known valid eimerian species have been given in Table I.

SUMMARY

Eimerian oocysts, recovered from faecal samples of 45 camel-calves, have been identified as belonging to three distinct species: *E. rajasthani*, *E. dromedarii* and *E. nölleri*. The previous work on eimerian species of camel has been reviewed and essential oocystic characteristics of the valid species tabulated.

TABLE I. OOCYSTIC CHARACTERISTICS OF VALID EIMERIAN SPECIES

Characters		*E. cameli*	*E. dromedarii*	*E. nölleri*	*E. pellérdyi*	*E. rajasthani*
Oocyst	Shape	Sub-spherical to spherical	Oval or sub-spherical	Oval	Oval or ellipsoidal	Ellipsoidal
	Size	23·3-34·3 μ × 20·6-30·2 μ, average 28·3 μ × 25·5 μ	23·1-32·5 μ ×19.9-25·2 μ, average 27·7 μ × 23·2 μ	80-100 μ × 65-80 μ	22·5-24 μ × 12-13·5 μ, mean 23·2 μ × 12·6 μ	35-39 μ × 25-27 μ mode 36 μ × 25 μ
	Polar cap	Absent	Present	?	Absent	Present
	Micropyle	Present	?(not visible in our material)	Present	Absent	Not visible
	Polar granule	Some times visible	Absent	Absent	Absent	Absent
	Oocystic residuum	Absent	Absent	Absent	Absent	Absent
Sporocyst	Shape	Lemon shaped	Oval or round	Elongated	Oval	Ovoid
	Size	15-17 μ×10 μ (Nöller) 12-14 μ×9-10·8 μ (Yakimoff)	8·5-10-5 μ×6·5-8·4 μ	?	9-10·5 μ×4·5-6·0 μ	14-15 μ×8-11 μ
	Sporocystic residuum	Present	Absent	Present (Tzygankov)	Present	Present
	Stieda body	?	Absent	?	Present	Present

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PATHOGENICITY OF EXPERIMENTAL INFECTION OF *SCHISTOSOMA INDICUM* MONTGOMERY (1906) TO YOUNG SHEEP

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SCHISTOSOMA INDICUM Montgomery (1906) is a common blood-fluke of domestic mammals in India. It infects the horse, donkey, camel, cattle, sheep, goat and rarely buffalo. Its reported occurrence in the zebra in Africa has been contradicted by Le Roux (1961). It is associated with nodulated hepatic cirrhosis in horses (Datta, 1933), sheep and goats (Rao, 1947). Investigations into various aspects of this important blood-fluke were carried out at this Institute under the auspices of the Indian Council of Agricultural Research. Studies on the morphology, life-history, host-specificity and biology have been reported earlier (Srivastava and Dutt, 1951, 1955a, b, 1962). A study of the effects of the infection of the parasite on young sheep is reported in this paper.

MATERIAL AND METHODS

Twelve lambs, born in the Institute's flock and reared on adequate ration, were divided into two identical groups after matching them for age, sex and body-weight. At the beginning of the experiment their age varied from five to seven months and body-weight from 16 to 37 pounds. The animals of one group were infected orally with 7,000-15,000 cercariae of *S. indicum*, obtained from experimentally infected specimens of *Indoplanorbis exustus*, in 9-26 daily doses of 200–1,300 cercariae per dose. The other group of six animals served as uninfected control. All the animals were allowed grazing on pastures frequented by other sheep and no attempt was made to control helminthic infections other than that of blood-flukes.

The animals were weighed once a month when off-feed for more than 12 hours. The following haematological observations were made fortnightly—determination of haemoglobin level, red blood corpuscular counts, and white blood corpuscular total and differential counts. The haemoglobin level was determined with a Zeiss-Ikon haemometer and the total blood cell counts with Spencer improved Neubauer haemocytometers using the diluting fluid and the technique recommended by Napier and Das Gupta (1942). The animals were examined daily for noting clinical symptoms. At the end of the experimental period of 18 months all the surviving animals of both the groups were sacrificed for autopsy.

RESULTS

Growth rate: The data of body-weight are presented graphically in Fig. 1. At the beginning of the experiment the mean values of the body-weights of the infected and control animals were 24·83 and 24·33 lb. respectively. At the end of the experiment

the mean values were 39·75 and 51·67 lb. respectively. Thus, in 18 months the mean gain in body-weight of the infected group was about 45 per cent less than that of the control group.

Haemoglobin level: The data of haemoglobin levels of the infected and control groups are shown graphically in Fig. 2. The mean haemoglobin levels of the infected and control groups at the beginning of the experiment were 10·67 and 10·93 gm. per 100 ml., respectively, and at the end of the experiment were 6·80 and 9·32 gm. per 100 ml., respectively. Thus, at the end of the experimental period, the mean haemoglobin level of the infected group was 27 per cent lower than that of the control and about 36 per cent lower than its own level at the beginning.

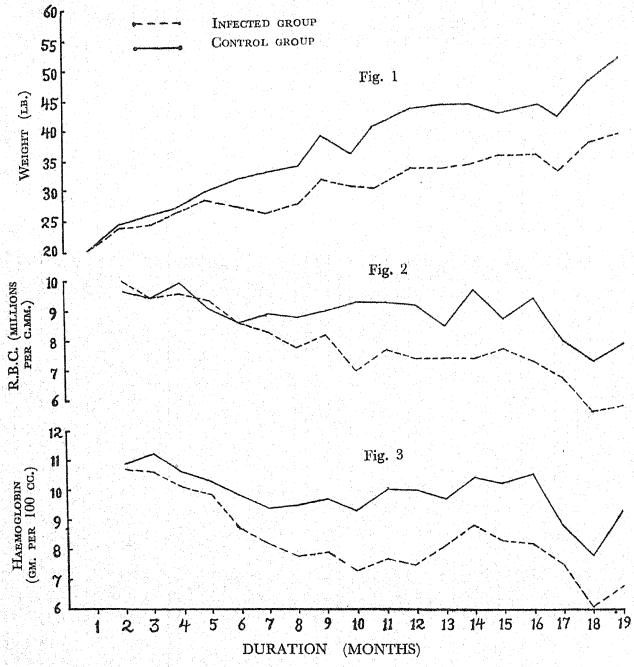

FIG. 1. BODY-WEIGHTS OF INFECTED AND CONTROL SHEEP
FIG. 2. HAEMOGLOBIN LEVELS OF INFECTED AND CONTROL SHEEP
FIG. 3. R.B.C. COUNTS OF INFECTED AND CONTROL SHEEP

Red blood corpuscular count: The mean values of red blood corpuscular counts of the infected and control groups at the beginning of the experiment were 9·99 and 9·75 million per c.mm., respectively, and at the end of the experiment 5·85 and 7·89 million per c.mm. respectively (Fig. 3). At the end of 18 months the red blood corpuscular count of the infected group was 25 per cent lower than that of the control and 41 per cent lower than its own count at the beginning.

White blood corpuscular counts: The mean counts of the infected and control groups for the total period of the experiment were 10·19 and 12·56 thousand per c.mm. respectively. In the infected group there was gradual rise in total counts up to the fifth month after infection and then a gradual but irregular fall until the last month of the experiment when the count was the highest. The total count of the control group was always higher than that of the infected group and it also reached the highest level during the last month of the experiment. The infected group had significantly higher eosinophils, significantly lower lymphocytes and non-significantly higher neutrophils.

The mean values of the body-weight and blood constituents of the infected and control groups for the total period of the experiment are given in Table I.

TABLE I. MEAN VALUES OF BODY-WEIGHT AND BLOOD CONSTITUENTS OF INFECTED AND CONTROL SHEEP

Constituents	Control		Infected		't'
	Mean	Standard error	Mean	Standard error	
W.B.C. (thousands/c.mm.)	12·56	0·208	10·19	0·241	HS
R.B.C. (millions/c.mm.)	9·02	0·155	8·01	0·152	„
Haemoglobin (gm./100 c.c.)	9·85	0·145	8·47	0·173	„
Lymphocytes (per cent)	49·43	0·943	46·82	1·104	S
Neutrophils (per cent)	40·56	0·972	41·60	1·082	NS
Eosinophils (per cent)	10·03	0·522	11·70	0·603	S
Body-weight (lb.)	37·47	1·064	30·38	0·854	HS

HS=highly significant; S=significant; NS=non-significant.

Other observations: There was no difference in the body temperature of the infected and control groups. At the beginning of the experiment there was no difference in the consistency of the faeces also, but during the later part of the experiment the infected animals passed diarrhoeic faeces more frequently than the controls. The only constant symptom of the infected animals was debility.

Mortality: Two infected animals died during the experiment and their death could be attributed to schistosomiasis. One of them died about ten and a half months after infection and the other after 15 months. During the last month of the experiment

one control animal died due to an internal abscess, resulting from an injury which outwardly appeared to have healed.

Autopsy findings: The data of recovery of schistosomes and of egg contents of the viscera of the infected animals are given in Table II.

TABLE II. DATA OF INFECTION OF EXPERIMENTAL SHEEP WITH *S. INDICUM*

Sl. No. of the animal	Total cercariae given	Age of infection at autopsy (months)	No. of schistosomes recovered	Eggs in viscera		
				Liver	Intestine	Lungs
1	7,000	18	335	+	+	0
2	7,000	18	nil	0	0	0
3	11,000	18	300	+	+	+
4	11,000	18	nil	+	+	0
5*	15,000	15	nil	+	+	0
6*	15,000	10·5	392	+	+	0

*Animal died.

One of the animals exposed to 7,000 cercariae was not infected at all. Its faeces remained negative throughout and at autopsy neither schistosomes nor their eggs could be detected in any of the organs. In two other cases the liver and intestinal wall showed heavy deposits of non-viable eggs, but no schistosome was present. Mature worms of both the sexes were recovered from the other three animals. The worms occurred in the mesenteric veins, liver and in one case in the lung also. The liver in the infected animals was cirrhotic and showed heavy deposits of eggs and haemosiderin pigments. Heavy deposits of eggs occurred in the intestinal wall, especially in the caecal wall. In one case the spleen also showed haemosiderin pigments. Both the infected and control animals harboured certain other helminthic infections. The mean numbers of such helminths recovered per animal of the two groups are given in Table III.

TABLE III. MEAN NUMBERS OF GASTRO-INTESTINAL HELMINTHS RECOVERED FROM EXPERIMENTAL AND CONTROL SHEEP

Species of helminth	Mean number recovered	
	Infected group	Control group
Cotylophoron cotylophorum	282	179
Stilesia globipunctata	4	3
Haemonchus contortus	156	57
Bunostomum trigonocephalum	1	<1
Oesophagostomum columbianum	80	93
Trichuris globulosa	26	25

DISCUSSION

Statistical analysis of the data on body-weight and blood constituents revealed that the infected group had highly significant retardation in weight gain, highly significant reduction in haemoglobin level, red and white blood corpuscular counts, significant increase in eosinophils, significant reduction in lymphocytes and non-significant increase in neutrophils. All these cannot be attributed to the schistosome infection alone, as the infected group had a slightly higher burden of gastro-intestinal helminths. However, this higher worm burden may be atrributed to the schistosome infection, since the two groups of animals were otherwise maintained under identical conditions.

The data obtained of red blood corpuscular counts and haemoglobin level of the control sheep are comparable to that reported by Murty (1957) for normal Indian sheep. But the values of total white blood corpuscular counts are higher, which indicate slight leukocytosis. We are unable to explain this fully. The formation of abscess in one animal is undoubtedly partially responsible for this.

The experimental animal, which was totally refractory to *S. indicum* infection, was one of the two receiving the lowest infective dose. The two infected animals which died of schistosomiasis were the ones receiving the highest infective dose. While one of them (No. 6) harboured a large number of schistosomes, the other (No. 5) had none at all. Another animal (No. 4) receiving the next lower dose was also negative for live worms at autopsy. Both these animals showed heavy deposits of eggs in the liver and intestinal tissues. Undoubtedly these two were cases showing the self-cure phenomenon. The factors responsible for self-cure in schistosomiasis are not definitely known. In these particular instances self-cure does not appear to be due to the natural death of the parasites, since the other animals examined at the same time or even later harboured living schistosomes. The fact that the self-cure occurred in animals receiving higher doses of the infection appears to indicate that it has been brought about by the host's immunological responses, as reported by Michel (1952) in case of *Trichostrongylus retortaeformis* infection in rabbits.

The mechanism of self-cure in schistosomiasis is the destruction of the parasites in the liver (and occasionally in the lung) where they are ultimately phagocytosed. As indicated by Michel (1954) in the case of *Dictyocaulus viviparus* infection in cattle self-cure may be detrimental to the host itself. He observed a significant connection of the fatal fog fever syndrome with self-cure of lungworm infection. In the present experiment also self-cure was connected with the death of one animal (No. 5). The other animal (No. 4) which had undergone self-cure became very ill at about the same time as the former died, but it could withstand the reaction of the self-cure mechanism.

As this experimental study would indicate, *S. indicum* appears to be highly injurious to sheep. In nature the incidence of this infection in sheep is very high (Dutt and Srivastava, 1963).

SUMMARY

An experiment was conducted to determine the pathogenicity of *Schistosoma indicum* infection to young sheep. Six animals were exposed to 7,000-15,000 cercariae and another six animals served as controls.

The infected group had higher mortality, highly significant retardation in weight gain, highly significant reduction in haemoglobin level, red and white blood corpuscular counts, significant increase in eosinophils, decrease in lymphocytes and non-significant increase in neutrophils.

The experiment also furnished evidence of occurrence of the self-cure phenomenon in schistosomiasis, the cause and mechanism of which have been discussed.

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STUDIES ON GESTATIONAL OESTRUS IN BOVINES

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OESTRUS is usually not evinced during gestational period in mammals. Its occurrence, however, in cattle has been reported by Schmid (1902), Strodthoff (1922), Bullard (1934), and Mirskaja and Smirnov-Ugrjumov (1941). Donald (1943) observed that oestrus occurred in dairy cows, during pregnancy at any time from 15-232 days, but most frequently in the first to third month, and that post-conception matings could occur in heifers and cows in any lactation. According to Nalbandov (1958) about 10 per cent of cows showed one or more heats during pregnancy and he considered that this figure might be even higher, if the pregnant cows were regularly checked for heat. Rahlmann and Mead (1958) analysed data pertaining to 3,159 cows from seven herds in the U.S.A. for the years 1935 to 1957 and found that oestrus following conception occurred 162 times in 147 gestation periods of 141 cows. The incidence of gestational oestrus in their studies was 2·07–7·75 per cent in different herds. Erb and Morrison (1958) found the incidence of oestrus after conception to be 5·62 per cent in 6,751 reproductive periods of Holstein–Friesian cattle.

Knowledge concerning the occurrence of gestational oestrus in Indian breeds of cattle is scanty. In the present study the incidence of gestational oestrus in Hariana cattle and Murrah buffaloes has been reported.

MATERIAL AND METHODS

The animals in the experimental Hariana and Murrah herds of the Indian Veterinary Research Institute, Izatnagar, have been under regular sexual health control since 1956. The occurrence of oestrus in the animals was detected with the help of vasectomised bulls which were allowed to run with the herds daily. The animals exhibiting signs of oestrus were investigated in detail for intensity and pattern of oestrus. Pregnancy was diagnosed clinically by rectal palpation method 40 to 45 days after artificial insemination. The animals showing oestrus during pregnancy were also clinically investigated and the intensity of oestrus and the stage of its occurrence during pregnancy were recorded.

RESULTS AND DISCUSSION

During the experimental period (1956-60) in all 312 oestruses in heifers, 1,369 in cows and 413 in buffalo-cows were observed. Fifteen oestruses in heifers, 104 in cows and 25 in buffalo-cows occurred during pregnancies. The incidence of the occurrence of gestational oestrus was 4·80, 7·61 and 6·05 per cent for heifers, cows

and buffalo-cows respectively. The details concerning the stage of pregnancy at which oestrus occurred and the range of variation are given in Table I.

TABLE I. INCIDENCE AND STAGE OF OCCURRENCE OF GESTATIONAL OESTRUS

	Total No. of oestruses observed	No. of gestational oestruses	Incidence (per cent)	Stage of gestational period at which oestrus occurred (days)	
				Mean	Range
Heifers	312	15	4·80	112·1 ± 17·0	18–259
Cows	1,369	104	7·61	113·2 ± 6·5	7–278
Buffalo-cows	413	25	6·05	108.4 ± 11.8	39–254

It will be observed from Table I that the oestrus occurred on an average at 112·1 days of pregnancy in heifers, 113·2 days in cows and 108·4 days in buffalo-cows.

The frequency distribution of the incidence of gestational oestrus (Table II) shows that the oestrus is usually evinced within 180 days of pregnancy in both cattle and buffaloes.

TABLE II. PERCENTAGE FREQUENCY DISTRIBUTION OF THE INCIDENCE OF GESTATIONAL OESTRUS

Interval (days)	Heifers		Cows		Buffalo-cows	
	Frequency	Per cent	Frequency	Per cent	Frequency	Per cent
1–30	1	6·7	13	12·5	0	0·0
31–60	2	13·3	14	13·5	5	20·0
61–90	4	26·6	14	13·5	8	32·0
91–120	1	6·7	6	5·7	3	12·0
121–150	1	6·7	19	18·3	3	12·0
151–180	5	33·3	19	18·3	3	12·0
181–210	0	0·0	8	7·7	1	4·0
211–240	0	0·0	10	9·6	1	4·0
241–270	1	6·7	1	0·9	1	4·0
Total observations	15		104		25	

The data pertaining to the intensity and recurrence of gestational oestrus during the period of gestation are presented in Table III. The intensity of oestrus was usually normal. One heifer, 12 cows and two buffalo-cows showed oestrus twice, and four cows three times during a single gestation.

TABLE III. INTENSITY AND RECURRENCE OF GESTATIONAL OESTRUS

	Heifers		Cows		Buffalo-cows	
	No.	Per cent	No.	Per cent	No.	Per cent
Total gestational oestrus	15	..	104	..	25	..
Intensity of gestational oestrus:						
Pronounced	1	6·6	2	1·9	0	0·0
Normal	14	93·4	75	72·1	22	88·0
Weak	0	0·0	27	26·0	3	12·0
Gestational oestrus evinced in single reproductive period:						
Twice	1	6·6	12	11·5	2	8·0
Thrice	0	0·0	4	3·8	0	0·0

Some traits of the breeding efficiency of both cows and buffaloes showing gestational oestrus were compared with their respective herd averages. The animal showing oestrus during pregnancy required on an average less number of insemination per pregnancy (Table IV). The age at conception in heifer showing gestational oestrus is comparatively much less. The interval between calving and fertile oestrus in both cow and buffalo is also less than the herd average.

TABLE IV. BREEDING EFFICIENCY OF ANIMALS SHOWING GESTATIONAL OESTRUS

	For animals showing gestational oestrus			Herd average		
	Heifer	Cow	Buffalo-cow	Heifer	Cow	Buffalo-cow
No. of A.I. per conception	1·60	1·57	1·38	1·68	1·83	1·71
Interval between calving and 1st oestrus (days)	1,126·9*	64·1	115·6	1,315·6*	84·9	123·0
Interval between calving and fertile oestrus (days)	1,297·2†	97·3	151·3	1,436·3†	138·9	158·1

*Interval between date of birth and appearance of 1st oestrus.
†Interval between date of birth and fertile oestrus.

It is generally believed that the oestrous cycles are suspended with the onset of pregnancy. The studies reported by several workers already mentioned earlier and the results obtained in the present work show that the oestrus symptoms are evinced in a large number of cases at any time during the period of gestation. The oestrus, however, is usually evinced once or twice during a single gestation period and the

pattern of its occurrence is not as regular as in non-gravid females. The intensity of oestrus has been found to be normal as in the regular oestrus pattern.

In natural mating cervical seal in pregnant animal is not likely to be disturbed and the chances of abortion as a result of mating are much remote. As recto-vaginal method is largely used for artificial insemination there is every chance of breaking through the cervical seal with the catheter, resulting in abortion of foetus. Of the cases of gestational oestrus studied in 47 per cent heifers, 40 per cent cows and 52 per cent buffalo-cows, oestrus had occurred within 90 days of pregnancy. This knowlege is of great practical significance to the workers engaged in the artificial insemination work.

During the initial stages of pregnancy there is considerable follicular development in ovaries. This may be the cause of occurrence of oestrus symptoms due to increased level of oestrogenic hormone. Hinze (1959) detected a high incidence of cystic ovaries before pregnancies in 71·3 per cent of the cows, showing oestrus during gestation. None of the animals in the present study had cystic degeneration of ovaries. During advanced pregnancy, though the placental oestrogen reaches much higher level, it is counteracted by progesterone and that is why the oestrus symptoms are not usually evinced during this period (Nalbandov, 1958).

Animals showing oestrus while pregnant are often considered as 'not in calf' by the breeder and are sometimes disposed of as sub-fertile. In fact, there is an indication that such animals are likely to have better breeding efficiency.

SUMMARY

Studies on gestational oestrus have been made in Hariana cattle and Murrah buffaloes.

Oestrus was found to occur in all stages of pregnancy but mostly within 180 days of gestation. The incidence of pregnancy heat was found to be 4·8, 7·6 and 6·1 per cent in heifers, cows and buffalo-cows respectively. The oestrus occurred on an average $112 \cdot 1 \pm 17$ days in heifers, $113 \cdot 2 \pm 6 \cdot 5$ days in cows and $108 \cdot 4 \pm 11 \cdot 8$ days in buffaloes after conception. The breeding efficiency of the animals showing gestational oestrus was found to be better than the herd average.

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FACTORS RESPONSIBLE FOR CANARY COLOURATION OF THE WOOL

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CANARY colouration in wool is a permanent yellow stain which develops in certain breeds of sheep during a particular season. This stain can only be removed by bleaching which, however, damages the wool fibre, thereby lowering the quality.

Various authors have given different causes for the yellow discolouration in wools. From their work it seems that the discolouration is caused: (i) by heat in the presence of moisture (Sidey, 1931; Lee, 1949; Duncan, 1957); (ii) by photochemical decomposition of wool (Saurer, 1916; Speakman and McMahon, 1939; McMahon and Speakman, 1941; Von Bergen and Mauersberger, 1948; Lundgren, 1955; Le Roux, 1959); (iii) due to the bacterial multiplication in the fleece (Anonymous, 1946b; Serra and De Matos, 1951); (iv) decomposition of wool due to oxidation (Cunliffe, 1936; Cronje, 1959); and (v) staining due to pigment in the suint (Sidey, 1931; Barker, 1931; Botha, 1945; Belschner, 1957; Poats and Fong, 1957; Lang, 1960).

About 30 per cent wool produced in India develops canary colouration (Narayan and Narayan, 1955). It is widespread especially in the northern plains which grow the world famous 'Vicaneri wools'. In this article various factors responsible for canary colouration have been studied.

MATERIAL AND METHODS

Thirty-six ewes and 12 rams, above one year in age, of Chokla type Bikaneri sheep were selected for this study. The ewes were not bred during the experimental period and a year prior to it. These were divided into three groups on the basis of their wool production estimated during a standardization period of 60 days, so that all the groups had the same average wool production per ewe. One of these groups received a daylight: darkness rhythm of 8 hours light: 16 hours darkness (L_1 group); another group a photoperiod of 16 hours light: 8 hours darkness (L_2 group); and the third group served as a control. The photoperiodic groups were housed in rooms with 40-watt daylight fluorescent tubes suspended from the roofing; temperature and humidity in these rooms were adjusted to vary as per the atmospheric conditions with the help of inlet ducts and exhaust fans. The control group was housed in a bamboo pen whose walls were screened with jute curtains in order to adjust the intensity of radiation to the level of the other two groups, i.e., 15 K cal./sq.m./hr. at sheep's eye level. The wool from six tattooed regions namely, withers, back, neck, shoulders, body and breech, on the left side of the ewes was shorn every 30 days.

The rams were paired on the basis of their live-weights and divided into two groups. They were housed in the same way as the control group of ewes. These were

shorn together with ewes in the middle of February, 1959. In the middle of the month of March, 1959 a patch of wool on the left side on the body of all rams was closely clipped with fine scissors and then shaved off with the help of sharp safety razor. On this portion a square chequer of the size of 10 cm. × 10 cm. divided into 25 small squares each of the size of 2 cm. × 2 cm. was tattooed. Two squares were selected by randomization for each pair of rams separately for wool sampling. All the rams were kept indoors in the shade for three months continuously (three periods of 30 days each); and later, one group of rams (Sun group) was exposed to direct sunlight for at least five hours daily, except on rainy days, by turning them out on pasture with the mouth-gags mounted. The other group (Shade group) of rams remained indoors throughout the experimental period.

All the animals were stall-fed and received their nutritive requirements as per Morrison's Feeding Standards for sheep. A few lambs (11) were also included in the study occasionally. Two of the rams died during the course of the experiment, as also one ewe from the control group. Two ewes from the L_a group got accidentally mated with a ram, hence, these ewes together with their trio mates were eliminated from statistical studies. The statistical analysis was conducted by the method of least squares (Snedecor, 1957).

(a) *Critical climatic conditions responsible for canary colouration, region of onset and sequence of progress:* For the study of critical climatic conditions responsible for the development of canary colouration, region of onset and the sequence of progress on the whole fleece, observations were made on 12 anatomically defined regions on the right side of both the rams and the ewes, at an interval of 28 days each. These regions were (Fig. 1) withers, back, loin, rump, neck, shoulder, body, breech, shoulder point, foreflank, belly and hindflank. However, this could not be continued at advanced stage of wool growth due to penetration of dirt in the fleece; hence, wool samples from tattooed regions on left side of ewes harvested monthly were utilised. The wool samples were scoured, oven-dried and conditioned to room atmospheric temperature and humidity before scoring. The ewes were afterwards shorn off their fleece in three instalments—August, September and October. Four ewes from each of the three groups were selected at random each month. Similarly, the rams were shorn in September and October. The pairs of rams to be shorn in each month were selected at random. After shearing the animals were washed with soap and running water and were again clipped with the help of a four-inch blade curved scissors leaving an uniform wool pile of not more than 2 mm. on their body and then washed with soap and running water. Besides the wool samples from the ewes as described earlier, samples from the tattooed patches on the mid-region of rams were utilised in the necessary determinations.

(b) *Variation in intensity of canary colouration on different body regions and between the sexes:* Previous to their autumn shearing wool samples, about 5 to 10 gm., were collected from the above-defined 12 body regions of all the ewes and the rams. Determination of the intensity of discolouration of wools was made by the following method: A standard set of wool samples were prepared by teasing and homogeneously mixing with a pair of hand cards to represent almost all the ranges in intensities of discolouration observed in the wool. These wool samples were graded as per the Munsell Colour notations which give the hue, value and chrome of a sample. The samples

were held directly behind the separate closest matching colour chips of the Munsell Soil Colour Charts (1954). The colours ranged from snow-white to golden yellow. The standard score prepared for the study is given in Table I.

TABLE I. STANDARD SCORE CARD TO STUDY DISCOLOURATION INTENSITY

Wool sample	Munsell notation	Score index
Snow-white	5 Y 8/N	1
White	5 Y 8/1·0	2
Dirty white	5 Y 8/1·5	3
Slightly yellowish	5 Y 8/2·0	4
Yellowish	5 Y 8/3·0	5
Pale yellow	5 Y 8/3·5	6
Yellow	5 Y 8/4·5	7
Dark yellow	5 Y 8/6·0	8
Golden yellow	3·75 Y 8/7·5	9

A fresh standard score card should be prepared every year, or a natural colour photograph transparency should be used, as the wool samples are likely to fade after constant use and exposure to light. The score method was found to be quick and accurate, and superior to the usual methods of colour estimation by Lovibond's colorimeter, photoelectric reflectometer and Leifo photometer which give innumerable troubles. With the score card index it was possible to present a total of 27 grades with + and − notations added to each score index to designate the intermediate grades of colour. However, in the present experiment only nine grades of colours (Table I) were used and the samples placed in the grade nearest to which it matched.

RESULTS

Critical climatic conditions responsible for the development of canary colouration, regions of onset and the sequence of progress of discolouration on the whole fleece: Observations made on the monthly wool samples from the six tattooed regions on the left side of ewes and those from the 12 regions on the right side of 59 animals have been summarised in Table II. By the middle of May (12-5-1959) the penetration of the dirt on the fleece was not deep enough and the objective colour estimation was thus possible to a fair degree of accuracy.

During the period May, 12 to May, 29 the development of discolouration was observed. Majority of the animals examined on the later date had stained fleeces at one region or the other, although the staining was only at the ends proximal to the skin. The weather conditions during that period were—air temperature 32·57°C and vapour pressure 13·60 mm. Hg., i.e., 37·5 per cent relative humidity. The staining

TABLE II. OBSERVATION ON THE ONSET OF CANARY COLOURATION

Period ending	Regions						Number of animals examined	Remarks
	Withers	Back	Neck	Shoulders	Body	Breech		
								(Period beginning from March 1st)
31-3-59	1	1	3	—	2	1	36	Adult ewes. Observations on left side.
24-4-59	8	2	10	10	4	10	36	Adult ewes. Observations on left side.
12-5-59	14	17	11	9	5	3	59	Observations on right side, on unscoured fleece on animals consisting of rams, ewes and lambs.
29-5-59	8	8	15	16	9	15	17	Adult ewes. Observations on left side.
18-6-59	36	36	36	36	36	36	36	Adult ewes. Observations on left side.

of samples from the same regions at the subsequent sampling, i.e., on June 18, was complete in all the animals. The climatic conditions during the later period were—air temperature 33·66°C and vapour pressure 20·51 mm. Hg., i.e., about 52·5 per cent relative humidity.

In Table III, the climatic conditions during August-September to November-December are indicated. In October-November wool samples from ewes shorn in August and September were partially stained on the upper portions of the staple. Samples from the ewes shorn in October were not stained. This difference may be due to the already stained surrounding fibres in the fleece of earlier shorn animals.

TABLE III. OBSERVATIONS ON STAINING AFTER THE AUTUMN SHEARING OF EWES

Period	Climatic conditions			Remarks on staining
	Air Temp. (°C)	Vap. Press. (mm. Hg.)	Rel. Hum. (per cent)	
August-September	29·58	25·33	82·0	Stimulated staining
September-October	27·69	22·08	81·5	
October-November	23·28	16·27	77·0	Did not stimulate general staining, although some ewes continued to grow discoloured wools on certain regions
November-December	17·68	10·93	73·0	

Thus, climatic conditions during August-September and September-October induced staining, while conditions during October-November and in subsequent periods were not conducive to canary colouration. Canary colouration was not observed in rams shorn even in September.

In certain ewes withers and back regions continued to grow somewhat discoloured wool for a long time, the intensity being more in withers as compared to that in the back region. After the onset of staining fibres which had already grown and which were white so far took up the stain. Thus, the stain traverses along the fibre length.

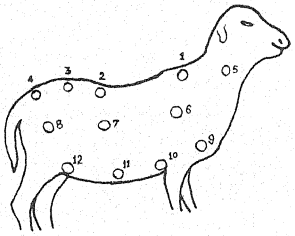

FIG. 1. TWELVE BODY REGIONS FOR THE DETERMINATION OF DISCOLOURATION OF WOOL

1. Withers 5. Neck 9. Shoulder point
2. Back 6. Shoulder 10. Foreflank
3. Loin 7. Body 11. Belly
4. Rump 8. Breech 12. Hindflank

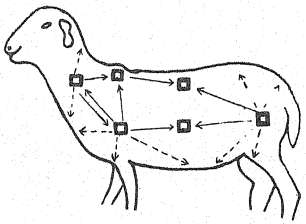

FIG. 2. REGIONS OF ONSET AND SEQUENCE OF PROGRESS OF CANARY COLOURATION

It appears from the data presented above that the onset of staining starts at the neck, shoulder and breech regions almost simultaneously, and from there it spreads to other parts of the fleece (Fig. 2). Preliminary observations made in autumn of the year 1958 also showed a similar trend.

Effect of different photoperiodic treatments on the discolouration score of wool in the ewes: These observations were based on the wool samples collected at the time of autumn shearing from ten trio groups of ewes. The data are presented graphically in Fig. 3. Analysis of variance of the data obtained is given in Table IV, from which it is seen that there was significant difference in intensity of discolouration due to the different photoperiodic treatments.

TABLE IV. ANALYSIS OF VARIANCE FOR THE DISCOLOURATION SCORE OF WOOL DUE TO DIFFERENT PHOTOPERIODIC TREATMENTS IN EWES

Source of Variation	D.F.	M.S.	F ratio
Groups (treatments)	2	1·576	40·410*
Regions	11	0·455	11·410*
Interaction (Groups × Regions)	22	0·039	

*$P < 0.001$

The critical difference values (Table V) show that there was significant difference in discolouration score of the samples from control group and the other two experimental groups.

TABLE V. AVERAGE DISCOLOURATION SCORE OF WOOL IN EWES DUE TO THE DIFFERENT PHOTOPERIODIC TREATMENTS

Groups	Av. discolouration score	C.D. values
Control	7·800	At 5% level 0·166
L_2	8·392	At 1% level 0·226
L_1	8·458	At 0·1% level 0·303

The means within the same bar are considered to be not different.

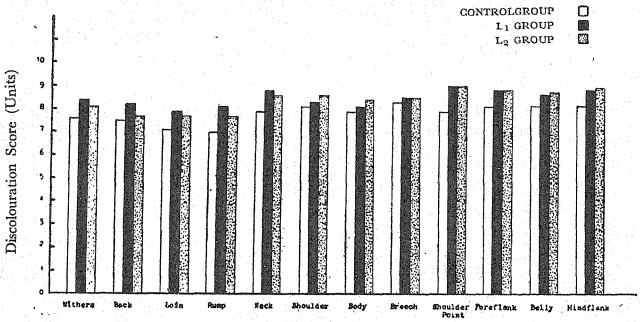

FIG. 3. DISCOLOURATION SCORE IN THE THREE GROUPS OF EWES

The effect of sun's direct radiation on the discolouration score of the wool in rams: Data on four rams from Sun group and their pair mates from the Shade group are presented graphically in Fig. 4. The analysis of variation for the data is given in Table VI.

TABLE VI. ANALYSIS OF VARIANCE FOR THE DISCOLOURATION SCORE OF WOOL DUE TO THE EFEFCT OF SUN'S DIRECT RADIATION

Source of variation	D.F.	M.S.	F ratio
Groups (treatments)	1	0·023	0·365 N.S.
Regions	11	0·536	8·492*
Error	11	0·063	

N.S.=Not significant *$p < 0·001$

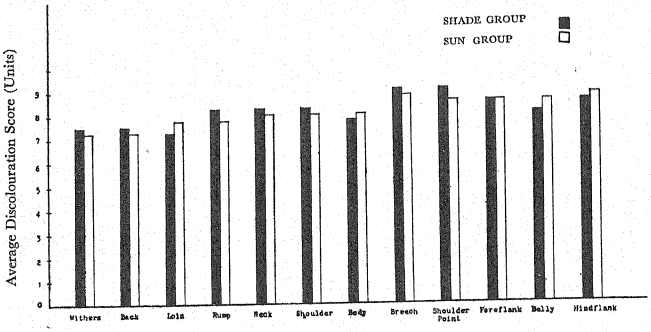

FIG. 4. DISCOLOURATION SCORE OF WOOL SAMPLES IN THE TWO GROUPS OF RAMS

There was no effect of the exposure to sun's direct radiation on the intensity of staining. However, there was difference in the intensity of discolouration of wool from the different body regions of the rams, same as, was in the case of the ewes.

Effect of shearing in different months on the intensity of discolouration of wool in ewes and rams: Twelve ewes, four from each of the three photoperiodic groups, were shorn in mid-August, mid-September and mid-October. Observations on 11 ewes belonging to the Control group only were considered for this study as the colouration was influenced by photoperiodicity. Three pairs of rams were selected at random for shearing in September, the remaining being shorn in October. As there was no effect of exposure to sun's direct radiation on the discolouration intensity of the wool, the available data on all the rams were pooled together. The data on the average discolouration score of wool for the ewes and the rams are summarised in Table VII.

TABLE VII. AVERAGE DISCOLOURATION SCORE OF WOOL FROM EWES AND RAMS (AVERAGE OF 12 BODY REGIONS)

Month of shearing	Range of discolouration score		Average for the month	
	Ewes	Rams	Ewes	Rams
August	7·33 to 8·08	..	7·77	..
September	7·00 to 8·08	6·00 to 8·33	7·52	7·50
October	6·91 to 8·67	7·25 to 8·67	7·94	8·06

The analysis of variance is summarised in Table VIII, from which it is seen that there was no difference in the intensity of discolouration of wool, both in the ewes and in the rams, due to the treatment of shearing in the different months.

TABLE VIII. ANALYSIS OF VARIANCE OF THE AVERAGE DISCOLOURATION SCORE OF WOOL IN EWES AND RAMS SHORN IN DIFFERENT MONTHS

Source of variation	Ewes			Rams		
	D.F.	M.S.	F ratio	D.F.	M.S.	F ratio
Months	2	0·160	0·478 N.S.	1	0·842	1·562 N.S.
Error	8	0·335		9	0·539	

N.S.=Not significant.

Variation in intensity of discolouration of wool between sexes: The data on the 11 rams and on the 11 ewes belonging to the Control group were statistically analysed to find the difference in intensity of discolouration between males and females. The data are presented graphically in Fig. 5. Analysis of variance (Table IX) indicated that the difference due to sexes was not significant whereas the difference due to regions was significant ($P<0·05$).

TABLE IX. ANALYSIS OF VARIANCE FOR THE DISCOLOURATION SCORES OF WOOL IN EWES AND RAMS

Source of variation	D.F.	M.S.	F ratio
Sexes	1	0·089	0·927 N.S.
Regions	11	0·376	3·917*
Error	11	0·096	

N.S.=Not significant. * $P < 0·05$

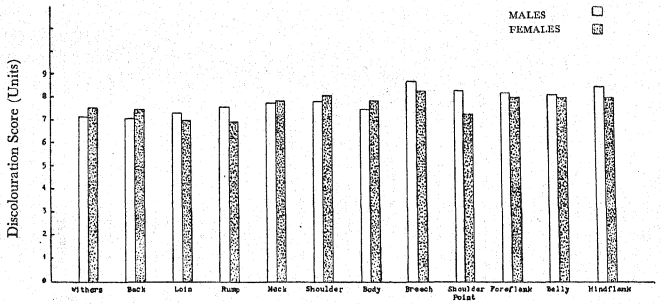

FIG. 5. DISCOLOURATION SCORE OF WOOL IN MALES AND FEMALES

Monthly variation in the intensity of canary colouration of wool: These observations were maintained on the wool samples obtained monthly from the side region of the rams. Data on the same four pairs of rams used in the experiment to find out the effect of sun's direct radiation were analysed statistically (Table X). The data are presented

TABLE X. ANALYSIS OF VARIANCE FOR THE INTENSITY OF DISCOLOURATION OF WOOL DURING THE COMPARATIVE PERIOD IN THE RAMS

Source of variation	D.F.	M.S.	F ratio
Pairs	3	2·481	12·594*
Months	8	77·681	394·320*
Treatments	1	0·222	1·127 N.S.
Pairs × Treatments	3	0·259	1·315 N.S.
Months × Treatments	8	0·160	0·812 N.S.
Pairs × Months	24	3·023	15·345*
Error	24	0·197	

N.S.=Not significant. *$P < 0.001$

graphically in Fig. 6. In order to find out whether there was any effect of the treatment of exposure to sun's direct radiation on the intensity of discolouration of wool, the data for the nine months' comparative period were first analysed. The analysis of variance is given in Table X from which it is seen that the treatment of exposure to sun's direct radiation had no effect of the intensity of discolouration. It was observed that the variations due to pairs (animals), months and interaction of pairs × months were highly significant. The interactions—pairs × treatments and months × treatments

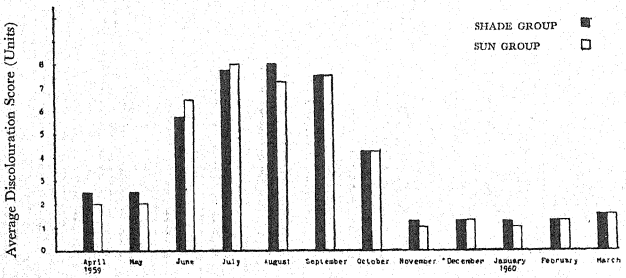

FIG. 6. DISCOLOURATION SCORE OF MONTHLY WOOL SAMPLES FROM MID-SIDE REGION OF RAMS

were not significant. On further testing the variance due to months against the variances due to interactions of months × treatments and pairs × months, the F values were found to be 485·506 and 25·697, respectively, both of which were highly significant ($P < 0·001$). Similarly, on testing the variance due to treatments against the variance due to interaction of months × treatments the F value obtained was 1·387, which was not significant. It seems, therefore, that exposure to sun's direct radiation has no effect on the intensity of staining of the wool samples from the side regions of rams. However, there was monthly variation in the intensity of staining, individual variation in the animals (pairs) and interaction between pairs × months. As there was no difference between the two groups (Sun and Shade) of rams, the data for these animals throughout the year were further pooled together. Analysis of variance is presented in Table XI from which it is seen that the variance due

TABLE XI. ANALYSIS OF VARIANCE FOR THE INTENSITY OF DISCOLOURATION OF WOOL THROUGHOUT THE YEAR IN THE RAMS

Source of variation	D.F.	S.S.	M.S.	F ratio
Pairs	3		1·816	6·013*
Months	11		63·829	211·354†
Pairs × Months	33		2·877	9·526†
Residual (error)	48		0·302	

*$P < 0·01$; †$P < 0·001$

to pairs, months, and their interaction were all highly significant. On further testing the variance due to pairs and months against the variance due to interaction of pairs × months it was seen that the F value for the former was less than 1 and, thus, not significant, whereas the F value for the months was 22·186 and was highly significant ($P < 0·001$). It is, thus, proved that there is monthly variation in the intensity of staining of wool, possibly due to the effect of climatic conditions. There was individual variation in the animals (pairs) and interaction between pairs × months. The critical difference values were calculated in order to find out if the differences between the intensity of discolouration were significant. The monthly average discolouration scores calculated from the observations on all the rams are presented in ascending order in Table XII.

Incidence of intensity of discolouration in different years: Six rams and six ewes selected at random were sampled in the previous year, i.e., in October, 1958. Except in the case of one ram and one ewe data on these were available for both the years 1958 and 1959. However, the ewes were subjected to different photoperiodic treatments in the year 1959 and it was seen that these treatments affected the intensity of discolouration of wool. The rams were also subjected to two different treatments in the year 1959, although in the latter case the treatments were found not to affect the intensity of

TABLE XII. DISCOLOURATION SCORE OF WOOL SAMPLES FOR DIFFERENT MONTHS

Months	Discolouration score	C.D. values
November	1·125	
January	1·125	
December	1·250	At 5% level 0·553
February	1·250	
March	1·500	
April	2·250	At 1% level 0·739
May	2·250	
October	4·250	At 0·1% level 0·964
June	6·125	
September	7·500	
August	7·625	
July	7·875	

Differences between the values under the same bar are not statistically significant.

discolouration of the wools. Nevertheless, the differences in the intensity of staining in the two years is obvious. In the autumn of the year 1958 the flock was kept indoors, whenever it rained, to avoid leaching of the fleeces. The data are presented in Table XIII from which it can be seen that there was a higher intensity of discolouration of wool in the year 1959. The onset of discolouration was in late June in the year 1958, whereas it was in late May in 1959. Hence, it may be concluded that there is an interaction between years × animals regarding the intensity of canary colouration of wool.

DISCUSSION

The time of onset of canary colouration depends mainly on the climatic conditions during different years. In the year 1959 the onset of discolouration was earlier by a month than in the year 1958. The actual climatic conditions responsible were 33°C air temperature and 14 mm. Hg. vapour pressure, i.e., 38 per cent relative humidity, or lower temperatures accompanied by higher relative humidities which were found to be air temperature around 28°C and 22 mm. Hg. vapour pressure, i.e., about 80 per cent relative humidity. Trials may be conducted in the psychrometric chamber to find the exact critical combinations of air temperature and humidity conducive to the development of discolouration. According to Hugo and Kroon (1958) 'Canary Colouration' developed when humidity is high and temperature above 40°C. Lang (1943, 1960) found that in the presence of suint and nearly saturated

TABLE XIII. INTENSITY OF DISCOLOURATION OF WOOL IN THE YEAR 1958 AND 1959

Tattoo No. of the animal	Average Discolouration Score		Remarks
	Year 1958	Year 1959	
Ewes			
110	7·909	7·750	Photoperiodic group C. in 59
185	6·182	8·167	„ „ L_1 „
187	6·545	8·000	„ „ L_2 „
215	7·000	7·833	„ „ C „
267	4·364	..	
273	4·200	7·000	„ „ L_1 „
Rams			
81	8·091	8·333	
103	7·000	8·166	
175	7·364	..	
221	5·727	8·667	
229	4·091	7·417	
264	4·636	6·000	

atmospheres, discolouration could be caused in wools at 35° to 50°C after ten days. Carter (1951) proved experimentally that suint secretion increased in high temperature condition, and that the secretions were higher under conditions of very high humidities. It may be recorded here that on very hot days in the month of May when the air temperature was appreciably high and the relative humidity quite low, the fleece of experimental sheep examined showed clear exudations from the skin.

Thus, at high temperature preferably accompanied by high humidities sheep sweat abundantly and suint output increases. The wool being highly hygroscopic the exudations are absorbed by the fibres. Again as suint further increases the hygroscopicity the fibres swell. Under these conditions, the secretions of the skin including the colouring pigment or pigments in the suint, if any, responsible for the development of ' Canary Colouration ', may be entering the fibres directly and treading along the fibre length, as the staple portion which had grown before the onset of staining was found to have the discolouration.

In winter or in periods such as spring, when the climatic conditions are contrary to those stated above the heat losses are diminished and, therefore, there would be negligible sweating and hence the suint secretions would be diminished. Carter (1951) also found that wax production increases in cold weather. Hence, it is logical

that the suint ratio is reduced and no staining takes place. The results of Freney (1940), wherein he observed that wool grown in summer have higher suint ratio than the wool grown in the winter, lend further support to the results obtained in this experiment.

A regular sequence of onset of stain on the body of sheep was found. The neck, shoulder and breech regions were found to develop the stain earliest. This may be due to higher metabolic activity at these regions and/or higher density of sweat glands with possibly higher sweating activity of the glands at these regions. Duerden (1927) stated that the metabolic activity at shoulder region may be higher than at other body regions. Narayan and Narayan (1955) reported that the staining starts first near the legs and then proceeds towards the mid-shoulder, mid-side and mid-breech and last of all on the back region.

The results presented here have shown that photoperiodic treatments in ewes produced differences in the intensity of staining, whereas exposure of rams to sun's direct radiation did not have any influence on the intensity of staining. Wool decomposition may take place due to continued exposure to light (Saurer, 1916; Sommer, 1927; Barritt and King, 1929; Smith and Harris, 1936; Von Bergen and Mauersberger, 1948; Le Roux, 1959). The discolouration in wools of Bikaneri rams was not due to photochemical decomposition, as exposure to sun's direct radiation did not produce any significant increase in the discolouration score. Besides, in the ewes, the Control group gave the lowest average discolouration score, followed by the L_2 group and lastly the L_1 group. There being no significant difference between L_1 and L_2 groups, the observed differences are postulated to be due to the air movement at the fleece level, the Control group of ewes being housed in a bamboo pen. This is further supported from the evidence obtained on rams wherein the 'Shade' group of rams confined to the pen had a higher intensity of discolouration than the Sun group which was let out daily. The air currents would be expected to be more freely circulating at the fleece level of the latter group of rams, thus facilitating the convective loss. Thompson (1953) stated that in fleece rot the openness of fleece is important and helpful in reducing the incidence. Thus, it seems that air currents also play some role in the intensity of staining of Bikaneri fleeces.

Difference in the intensity of discolouration between the sexes was found to be statistically not significant.

The present results show variation in the intensity of discolouration from region to region on the body of the animal. The highest discolouration score was observed on breech (Region 8) and the hindflank both in the rams and the ewes. Sampling from breech region is, therefore, considered adequate, especially since this region has also early onset of the stain.

Variations in the intensity of canary colouration may be due to the variation in genetical constitution of the animal and gene $\times$ environment interaction. The pairs (animals) $\times$ months interactions in this experiment were found to be highly significant. Variations in amount of suint secreted may be due to heredity (Hugo and Kroon, 1958). The quantity of suint may also vary from fleece to fleece (Barker, 1931; Belschner, 1957). Thus, individual variations in animals are to be expected. In fact these variations offer a chance for selection against the incidence of discolouration.

Anonymous (1946a) and Sobti (1959) have also mentioned about the variations in the intensity of canary colouration in various types of Bikaneri wools.

SUMMARY

'Canary Colouration' is induced in Bikaneri wools at 33°C air temperature and 14 mm. Hg., vapour pressure or at 28°C air temperature and 22 mm. Hg. vapour pressure.

The onset of discolouration is on neck, shoulder and breech regions almost simultaneously, and from there it spreads to other parts of the fleece. There are also variations in the intensity of discolouration on the fleece of the animal, the breech region showing the highest intensity. Hence, sampling on breech region for determination of discolouration is recommended.

No difference in intensity of staining is observed between the sexes.

Photochemical decomposition was not found to be the cause of canary colouration.

No effect has been found in the intensity of discolouration of wool due to shearing in different months in the autumn season.

ACKNOWLEDGEMENTS

The authors desire to thank the Director, Indian Veterinary Research Institute, Izatnagar, for providing facilities and his interest in the work. During the course of the experiment the senior author was recipient of Senior Research Fellowship in Animal Husbandry awarded by the Indian Council of Agricultural Research.

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ABSTRACTS

Effect of nutrient density and protein-energy inter-relationships on reproductive performance of the hen. TOUCHBURN, S. P. and NABER, E. C. 1962. *Poult. Sci.* **41:** 1481-88.

The term nutrient density was introduced during the present study to describe the concentration of known nutrients in relation to the total weight of ration. The nutrient density of rations fed to Leghorn laying-pullets was varied from 16 per cent protein and 960 calories of productive energy per pound at a calorie-protein ratio of 60. In all rations the animal protein, vitamins and minerals were provided in proportion to the total protein. The rate of egg production was not significantly affected by the nutrient density of the ration. A calorie-protein ratio of 80, in a 12 per cent protein ration caused a decrease in egg production. The efficiency of feed utilization increased as the nutrient density (concentration of known nutrients per pound) increased. The reduction in relative efficiency with the highest nutrient density ration (20 per cent protein, 1,200 cal./lb. productive energy), was attributed in large measure to over-consumption. It was observed that the energy and protein levels in the laying ration should be adjusted to permit a daily intake of at least 17 gm. of protein per bird for maintenance of 72 per cent egg production.

In all experiments fertility and hatchability were maintained at an exceptionally high level. The average fertility was 94 per cent and the average hatchability was 90 per cent. These characteristics provide very sensitive criteria of the nutritive adequacy of the experimental diets, especially with regard to vitamin and mineral content.—(S. B.)

Relationship between Hog-cholera and virus diarrhoea virus of cattle. (27199). SHEFFY, B., COGGINS, L. and BAKER, J. A. 1962. *Proc. Soc. exp. biol. Med.* **109:** 349-52.

The authors have presented the concept of resistance or protection having no direct immunological basis by the use of cattle virus diarrhoea (VD), virus against hog-cholera (HC) virus in the same way as Jenner used cow-pox virus vaccine to protect human beings against small-pox virus, immunologically related to each other.

In their repeated three sets of experiments using lots of litters of pigs and calves against two strains of VD virus—Oregon C, 24V and New York, 1 and one strain of HC virus—serum samples were collected before and after infecting or inoculating with the respective strain of virus and after challenge at 4, 7, 10, 14 and 21 days.

By serum neutralization tests with the tissue culture antigens it was observed that pigs that were initially given a dose of VD virus, though revealed before challenge a low antivirus diarrhoea titre of 27 at 28 days, showed an anti hog-cholera titre of 515 at 21 days after challenge with HC virus. The titre against VD also rose from 27 to 729.

In the reciprocal tests calves initially inoculated with HC virus showed a low titre of HC antibodies; but it rose to a peak parallel to VD antibody titre of 988 at 21 days, starting from the fifth day after challenge with the virulent VD virus.

Serum samples from calves that had not been given HC virus showed VD antibodies from the 11th day after inoculation with VD virus and rose to a titre of 720 at 21 days stage.

From this the authors have concluded that: (1) VD virus protects pigs against a lethal dose of HC virus; (2) the two viruses failed to share a common neutralizing antibody; (3) the VD virus appears to create a stage of secondary response to HC virus, which is otherwise fatal to them; this stage resembles the effect induced by a single inoculation of homologous inactivated viral antigen; and (4) the HC virus though apparently failed to give absolute solid immunity against VD virus, definitely indicated the effect of secondary response.—(M. N.).

REVIEWS

PATHOLOGIE DU LAPIN (in French). LESBOUYRIES, G. 1963. Librairie Maloine, Paris. pp. 227, 62 figs. Price: Paperbound 38 F.; Cardbound 48 F.

The book (Pathology of Rabbit) is divided into three parts. Part I deals with troubles connected with reproduction. In Chapter one the author discusses physiology of the sexes, causes of sterility in male and female, the accompanying troubles in infecundation, sexual desire, obstacles in coitus due to deformity causes by diseases in sex organs; troubles associated with fecundation; abnormal gametogenesis, faulty function of male and female organs; troubles of nidation and gestation; nutritional obstacles and those by natural infections, parturition and allayment.

The pathology of the newborn is dealt with in Chapter two, with reference to congenital malformations and infections.

Part II is restricted to pathology of the lapereaux and the following aspects have been treated: nutritional troubles, maladies caused by parasites; protozooses and helminthoses; this is followed by a note on troubles originating from infections.

Common maladies of rabbits are grouped under Part III. Those due to ultravirus are treated first, being followed by those due to bacteria. Neoplasmic infections, neither ultravirus nor neoplasmic, diseases caused by acid resistant bacteria, gram-negative bacteria and gram-positive bacteria are also described.

Symptoms, diagnosis, prognosis, treatment, prophylaxis (and list of pertinent references) are given for each disease. In some cases modes of infection and pathology of the agent are also included.

Affections of individual organs are dealt with in Part IV. Affections in paws caused by arthropod and champignon, those of the mouth, ear, digestive tract; stomach, intestine, coprophagus, etc., respiratory tract, etc., are treated and illustrated.

Part V is restricted to tumors and VI to intoxications.

Pertinent literature is cited at the end of each major topic treated.

The *Pathologic du Lapin* is very useful and instructive book on diseases of rabbit. It is authoritative and designed to provide research workers and those interested in animal production with a single comprehensive information source on the subject.—(P. K.)

PREVENTIVE VETERINARY MEDICINE. WHITE, E. G. and JORDAN, F. T. W. 1963. Brailliere, Tindall and Cox, London. pp. 334. Price 30s.

With the advent of faster means of transport and consequent movement of animals in larger numbers from one country to another, diseases have been provided with considerable scope to break their bounds. Export of frozen meat, deep-frozen semen and biological products are also responsible for the spread of diseases. This has naturally caused a swing away from therapy towards prophylaxis directed at countries, continents and regions. In the book *Veterinary Preventive Medicine* two eminent

veterinarians, E. G. White and F. T. W. Jordan, have focussed our attention on the importance of preventing diseases in animals. They have rightly stressed the fact that greater the knowledge of the cause and natural history of a disease, the better are the chances of controlling it. Part I of the book deals with this aspect. Herein are described the differences between 'disease' and 'health', as well as disease-causing factors and the resistance of the hosts. The epidemiology of types of diseases have been presented under those caused: (1) essentially by primary intrinsic factors, and (2) primarily by extrinsic factors. The spread of diseases and the methods for controlling them have been also dealt with.

Part II deals with the control of different types of diseases: infectious diseases, metabolic and hormonal diseases, behavioural disorders, diseases caused by non-living agents, viral diseases, bacterial disease, etc., each being discussed separately. Beginning with a brief introductory account of each disease the authors describe a few examples which illustrate different types of epidemiology and control, or are of sufficient magnitude to cause economic loss to the affected or in-contact animals. The use of preventive medicine to control diseases is described under each heading.

In Part III are described human, climatic and geographical features which influence control of diseases. This is a very interesting Section. The authors have tried to impress upon the readers that a stable Government and other political factors play an important role in preventing the occurrence of diseases. They have also discussed, with examples, the influence of economic and social factors. The necessity for establishment of organizations to produce biological products and employment of specialists to carry out diagnostic service is also emphasized. The activities of a few organizations are described to support this view.

Part IV is restricted to the discussion on bearing of diseases of domestic animals on human being, from both economical and public health angles.

The authors have dealt with the subject in the light of conditions prevailing in Britain. This does not lessen the value of the publication, as with slight changes the material can be made applicable to conditions in other countries. However, the value of the book would have been enhanced if details were given regarding: (i) important diseases such as blue-tongue, African horse-sickness, African swine-fever, which, having broken their natural boundary, are creating havoc in other countries, and (ii) the functioning of a number of international organizations playing important role in this subject.

The authors deserve to be complimented for treating the subject in a simple and impressive language. This book must be possessed by every veterinarian. The get-up of this book is good.—(R. R. L.).

MICROBIAL SENSITIVITY OF STRAINS OF *BRUCELLA* ISOLATED FROM HUMAN AND ANIMAL SOURCES IN INDIA

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In India so far no work has been carried out to study the sensitivity of local strains of *Brucella* isolated from animal and human sources to various drugs, and to compare their reactions with those observed in other countries. In this paper it is proposed to record the studies carried out on the microbial sensitivity of 36 strains of *Brucella* isolated from different parts of India from animal and human sources against 32 different chemotherapeutic agents, including various antibiotic and sulfonamide compounds. The paper-disc method (Murty and Makholia, 1963) was used for this screening, and the cultural, biochemical and serological characters of each strain were studied side by side.

MATERIAL AND METHODS

Strains of Brucella: The following 36 strains of *Brucella* including 25 *Br. abortus*, five *Br. melitensis*, three *Br. suis* and three *Br. intermedia* (Renoux, 1952) were subjected to the sensitivity tests.

(i) Strain Nos. 616/47, 611, 10/G31 and 17/G28 were recently isolated in this laboratory from cases of brucellosis at State Livestock Farm, Madhurikund, and College Dairy Farm, Mathura.

(ii) Strain Nos. F49, V19, F37, F31, Hissar, Bombay, *Br. suis* and *Br. melitensis* were received from the Director, Indian Veterinary Research Institute, Mukteswar-Kumaon, U.P.

(iii) Strain Nos. 8, 30, 45, 186, 341, 632, 799, 867, 879, 887, 900, 924, 932, 939, 974, 989, 997, B2, B36, AG, NL, SC, YPS and MP were received from Dr T. N. Mathur, Assistant Bacteriologist, Karnal, Punjab.

All the strains were subjected to the usual biochemical and serological tests comprising CO_2 dependence, H_2S production, sensitivity to thionine and basic fuchsin, agglutination with high titre serum and mono-specific sera and smoothness of the colonies. The procedure adopted at the Central Veterinary Laboratory, Weybridge, England (Anonymous, 1953) was followed in conducting and interpreting these tests and the results are incorporated in Table II.

Antibiotics and drugs: Thirty-two chemotherapeutic agents including 22 antibiotics were used for the sensitivity tests and except Staphcillin, (Methicillin), which was used at one concentration of 5 μg. per ml., all other drugs were tried at two concentrations. These drugs include: Penicillin and Bacitracin at 2 and 10 units per ml. concentration; Ilotycin (Erythromycin) at 2 and 15 μg. per ml. concentration;

Chlortetracycline (Aureomycin), Kanamycin (Kantrex), Oxytetracycline (Terramycin), Chloramphenicol (Chloromycetin), Neomycin (Mycifradin), Novobiocin (Cathomycin), Dimethychlorotetracycline (Decolomycin), Nitrofurantoin (Furadantin), Aerosporin (Polymyxin B) and Vancocin (Vancomycin) at 5 and 30 μg. per ml. concentration; Dihydrostreptomycin, Streptomycin, Oleandomycin (Matromycin), Viomycin sulphate and Colistin sulphate (Colymycin) at 2 and 10 μg. per ml.; Isonicotinic acid Tydrazide at 1 and 25 μg. per ml. concentration; Mycostatin Nystatin) at 25 and 100 units per ml. concentration; Sulfisomidine (Elkosin), Sulfisoxazole (Gantrisin), Sulfamethylthiadiazole (Thiosulfil), Sulfadiazine, Sulfathiazole, Sulfamethoxypyridazine, Triple sulfa (Sulfadiazine, Sulfamethazine and Sulfamerazine) and 2, 4-dimethoxy-6-sulfanilamide-1, 3-diazine (Madribon) at 50 and 300 μg. per ml. concentration; Mandelamine (Methenamine) at 1 and 3 μg. per ml. concentration; and Altafur and Staphcillin.

Paper discs containing the respective drug in the specified concentration supplied by Messrs Difco Laboratories, Detroit, Michigan, U.S.A., were used.

Technique: Plates of tryptose agar were prepared by pouring 15 to 20 ml. of sterile medium into petri-dishes of 95 mm. diameter. Each strain of the organism after testing its purity and characters was sown on tryptose broth and 24-hour-old culture was used to inoculate the plates. Two millilitres of the culture was added to each plate and was spread over the entire surface of the medium. The excess of fluid was discarded and the plates were then kept in the incubator at 37°C for half an hour for drying the surface of the medium. The paper discs containing the respective chemotherapeutic agents

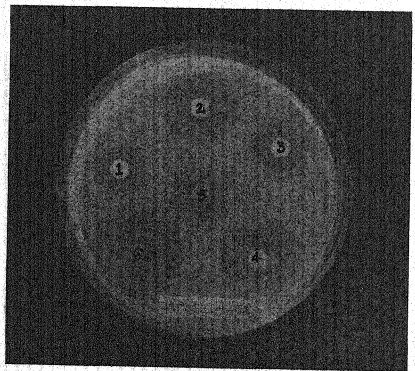

FIG. 1. MICROBIAL SENSTIVITY TEST WITH *Br. melitensis* (I.V.R.I.) ROUGH STRAIN

INDEX

1. STREPTOMYCIN LOW CONCENTRATION.
2. STREPTOMYCIN HIGH CONCENTRATION.
3. DIHYDROSTREPTOMYCIN LOW CONCENTRATION.
4. DIHYDROSTREPTOMYCIN HIGH CONCENTRATION.
5. PENICILLIN LOW CONCENTRATION.
6. PENICILLIN HIGH CONCENTRATION.

were placed over the plate at a distance of about 3 to 4 cm. apart (Fig. 1). All the plates were incubated at 37° C for 72 hours and the zones of inhibition were examined. If the zone of inhibition was more than 10 mm. in diameter in both dilutions it was considered to be 'highly sensitive' (++); if the zone of inhibition was up to 10 mm. and below, then as 'sensitive' (+); and if there was no zone of inhibition, then as 'resistant' (R). Depending on the reaction at both the concentrations of the drug, the strain was adjudged as 'highly sensitive', 'sensitive', 'slightly sensitive' or 'resistant'. The tests were replicated for four times and identical results were obtained.

RESULTS

All the 36 strains were highly sensitive to six antibiotics, viz., Chlortetracycline (Aureomycin), Dimethychlorotetracycline (Declomycin), Tetracycline (Achromycin), Kanamycin, Oxytetracycline (Terramycin) and Mandelamine (Methenamine). They were resistant to the following 16 drugs: Bacitracin, Colistin sulphate (Colymycin), Oleandomycin, Vancocin (Vancomycin), Viomycin sulphate, Staphcillin (Methicillin), Isonicotinic Acid, Tydrazide, Sulfisomidine (Elkosin), Sulfisoxazole (Gantrisin), Sulfamethylthiadiazole (Thiosulfil), Sulfadiazine, Sulfathiazole, Sulfamethoxypyridazine, Triple sulfa (Sulfadiazine, Sulfamethazine and Sulfamerazine), 2, 4-dimethoxy-6-sulfanilamide-1, 3-diazine (Madribon) and Mycostatin. The results of sensitivity were variable with the remaining ten chemotherapeutic agents. Table I shows the results of sensitivity of 36 strains to these ten drugs. The biochemical and serological characters of each strain are recorded in Table II. It would be evident from Table II that 25 of these strains showed biochemical and serological characters of *Br. abortus*, five of *Br. melitensis*, three of *Br. suis* and three of *Br. intermedia*. Two of the strains of *Br. melitensis* were rough variants. There was no significant difference in the drug sensitivity of rough strains from that of smooth strains (Table I). Three of the strains of *Br. abortus* were of buffalo-origin and they behaved similar to other strains of this organism in their serological and biochemical characters as well as in their drug sensitivity.

DISCUSSION

Most of the studies on sensitivity of strains of *Brucella* so far reported in foreign countries have been carried out by serial dilution method, while in this report the paper-disc method was used for carrying out these tests. Although 36 strains of *Brucella* were tested against 32 different chemotherapeutic agents including most of the new antibiotics, there were only six antibiotics to which all the strains were highly susceptible. These drugs were mostly broad spectrum antibiotics including Chlortetracycline, Dimethychlorotetracycline, Oxytetracycline, Tetracycline, Kanamycin and Mandelamine. Clinical trials with most of these antibiotics for treatment of animal brucellosis are not available and are, therefore, indicated. Out of the 32 drugs tried 16 were ineffective, while the reactions in case of ten of them were variable. Among the drugs which were ineffective include all the Sulfonamide compounds. Although Hamann and Huddleson (1939) observed that Sulfapyridine had some bacteriostatic effect *in vitro* on *Br. abortus*, clinical trials (Huddleson, 1943) showed that Sulfonamides were

TABLE I. SENSITIVITY OF *BRUCELLA* STRAINS TO THE DRUGS WHICH SHOWED VARIABLE REACTIONS

Strain of *Brucella*	Species or variant	Chloramphenicol		Dihydrostreptomycin		Streptomycin		Altafur		Nitrofurantoin		Aerosporin		Ilotycin		Penicillin		Neomycin		Novobiocin	
		High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low
611	*Br. abortus*	R	R	++	R	++	R	++	++	++	++	++	R	R	R	R	R	++	++	++	++
616/47	*Br. abortus*	+	R	++	+	+	R	++	++	++	++	++	+	R	R	R	R	++	++	++	++
10/G31	*Br. abortus*	+	R	++	+	++	+	++	++	++	++	R	R	R	R	R	R	++	+	++	+
17/G28	*Br. abortus*	+	R	++	+	++	R	++	++	++	++	R	R	R	R	R	R	++	+	++	++
F49	*Br. melitensis*	+	R	++	+	++	+	++	++	++	++	++	+	R	R	R	R	R	++	++	++
Hissar	*Br. melitensis*	++	++	++	++	++	++	++	++	++	++	+	R	++	R	++	+	++	+	++	++
Br. melitensis	*Br. melitensis*	++	++	++	+	++	+	++	++	++	++	+	R	++	R	R	R	++	+	++	+
F31	*Br. suis*	++	++	++	+	++	+	++	++	++	++	+	R	++	R	R	R	++	+	++	++
F37	*Br. suis*	++	++	+	R	+	R	++	++	++	++	R	R	++	+	R	R	++	+	++	+
Br. Suis	*Br. suis*	++	++	++	++	++	++	++	++	+	R	+	R	R	R	+	+	++	+	++	+
V19	*Br. abortus*	++	+	++	+	++	+	++	++	++	++	R	R	R	R	R	R	++	+	++	+
Bombay	*Br. abortus*	++	+	++	+	+	R	++	++	++	++	++	+	+	R	R	R	++	+	++	+
939	*Br. abortus*	++	+	++	++	+	R	++	++	++	++	+	R	R	R	R	R	++	+	++	+
974	*Br. abortus*	++	+	++	++	++	+	++	++	++	++	+	R	R	R	R	R	+	R	+	R
932	*Br. abortus*	++	+	+	R	+	R	++	++	+	+	+	R	R	R	R	R	+	R	+	R
924	*Br. abortus*	+	R	++	+	+	R	++	+	+	+	+	R	R	R	R	R	+	R	+	R
45	*Br. abortus*	+	R	+	R	+	R	++	+	R	R	+	R	R	R	R	R	++	+	+	R
186	*Br. abortus*	++	+	+	R	+	R	++	+	R	R	+	R	R	R	R	R	+	R	+	R
900	*Br. abortus*	++	+	+	R	++	+	++	+	++	+	+	R	R	R	R	R	+	R	+	R

TABLE 1 (*Concld.*)

Strain of *Brucella*	Species or variant	Chloramphenicol		Dihydrostreptomycin		Streptomycin		Altafur		Nitrofurantoin		Aerosporin		Ilotycin		Penicillin		Neomycin		Novobiocin	
		High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low
867	*Br. abortus*	++	+	+	R	++	+	++	++	+	R	+	R	R	R	R	R	+	R	+	R
997	*Br. abortus*	++	+	++	+	++	+	++	++	+	R	+	R	R	R	R	R	+	R	+	R
30	*Br. abortus*	++	+	+	R	++	R	++	++	+	R	+	R	R	R	R	R	++	R	++	R
799	*Br. abortus*	++	+	+	R	+	R	++	++	+	R	+	R	+	R	R	R	++	R	++	+
8	*Br. abortus*	++	+	+	R	+	R	++	++	++	+	+	R	R	R	R	R	++	+	++	+
341	*Br. abortus*	++	+	++	+	++	+	++	++	++	+	+	R	R	R	R	R	++	R	++	+
887	*Br. abortus*	++	+	+	R	+	R	++	++	+	+	+	R	R	R	R	R	+	R	+	R
632	*Br. abortus*	++	+	++	+	++	+	++	++	++	+	++	R	R	R	R	R	++	R	++	R
879	*Br. abortus*	++	+	+	R	+	R	++	++	+	R	+	R	+	R	R	R	+	R	+	R
989	*Br. abortus*	++	+	++	++	++	+	++	++	++	+	R	R	R	R	R	R	++	+	++	+
B2	*Br. abortus*	++	+	++	++	++	+	++	++	++	+	R	R	R	R	R	R	++	+	++	+
B36	*Br. abortus*	++	+	++	+	++	+	++	++	++	+	+	R	R	R	R	R	++	+	++	+
SC	*Br. melitensis*	+	R	++	+	++	+	+	R	+	R	+	R	R	R	R	R	++	+	++	+
NL	*Br. melitensis*	++	+	++	++	++	+	++	++	++	++	R	R	R	R	R	R	++	+	++	+
AG	*Br. intermedia*	++	+	++	++	++	+	++	R	++	R	+	R	R	R	R	R	++	R	++	+
YPS	*Br. intermedia*	++	+	++	++	++	+	++	R	++	R	+	R	R	R	R	R	++	+	++	+
MP	*Br. intermedia*	++	+	++	+	++	+	++	R	++	R	+	R	R	R	R	R	++	+	++	+

TABLE II. BIOCHEMICAL AND SEROLOGICAL CHARACTERS OF THE STRAINS OF *BRUCELLA* USED IN THIS STUDY

Strain of *Brucella*	Origin of the strain		CO_2 requirements	H_2S production (in days)	Smooth (S) Rough (R)	Growth in the presence of		Agglutination high with titre serum	Agglutination with mono-specific serum		Species or variant
	Host	Area				Thionin	Basic fuchsin		*abortus*	*melitensis*	
611	Cow	Mathura	—	2–3	S	—	+	+	+	—	*Br. abortus*
616/47	Cow	Madhuri-kund	+	1–3	S	—	+	+	+	—	*Br. abortus*
10/G31	Buffalo	,,	+	1–3	S	—	+	+	+	—	*Br. abortus*
17/G28	Cow	,,	—	1–3	R	—	+	+	+	—	*Br. abortus*
F49	—	I.V.R.I.	—	N	S	+	+	+	—	+	*Br. melitensis*
Hissar	—	I.V.R.I.	—	N	S	+	+	+	—	+	*Br. melitensis*
Br. melitensis	—	I.V.R.I.	—	N	R	+	+	+	—	+	*Br. melitensis*
F31	—	I.V.R.I.	—	4–5	S	+	—	+	+	—	*Br. suis*
F37	—	I.V.R.I.	—	4–5	S	+	—	+	+	—	*Br. suis*
Br. suis	—	I.V.R.I.	—	4–5	S	+	—	+	+	—	*Br. suis*
V19	—	I.V.R.I.	—	4	S	—	+	+	+	—	*Br. abortus*
Bombay	—	I.V.R.I.	+	2–3	S	—	+	+	+	—	*Br. abortus*
939	Cow	Punjab	+	1–3	S	—	+	+	+	—	*Br. abortus*
974	Cow	Punjab	—	2–3	S	—	+	+	+	—	*Br. abortus*
932	Cow	Punjab	+	2–3	S	—	+	+	+	—	*Br. abortus*
924	Cow	Punjab	+	2–3	S	—	+	+	+	—	*Br. abortus*
45	Cow	Punjab	+	2–3	S	—	+	+	+	—	*Br. abortus*
186	Cow	Punjab	+	2–3	S	—	+	+	+	—	*Br. abortus*
900	Cow	Punjab	+	2–3	S	—	+	+	+	—	*Br. abortus*
867	Cow	Punjab	+	2–3	S	—	+	+	+	—	*Br. abortus*
997	Cow	Punjab	+	1–3	S	—	+	+	+	—	*Br. abortus*
30	Cow	Punjab	+	2–3	S	—	+	+	+	—	*Br. abortus*
799	Cow	Punjab	+	2–3	S	—	+	+	+	—	*Br. abortus*
8	Cow	Punjab	+	3	S	—	+	+	+	—	*Br. abortus*
341	Cow	Punjab	+	2–3	S	—	+	+	+	—	*Br. abortus*
887	Cow	Punjab	+	2–3	S	—	+	+	+	—	*Br. abortus*
632	Cow	Punjab	+	2–3	S	—	+	+	+	—	*Br. abortus*

TABLE II (*Concld.*)

Strain of *Brucella*	Origin of the strain		CO_2 requirement	H_2S production (in days)	Smooth (S) Rough (R)	Growth in the presence of		Agglutination with high titre serum	Agglutination with mono-specific serum		Species or variant
	Host	Area				Thionin	Basic fuchsin		*abortus*	*melitensis*	
879	Cow	Punjab	+	2–3	S	—	+	+	+	—	*Br. abortus*
989	Cow	Punjab	+	2–3	S	—	+	+	+	—	*Br. abortus*
B2	Buffalo	Punjab	+	1–3	S	—	+	+	+	—	*Br. abortus*
B36	Buffalo	Punjab	+	1–3	S	—	+	+	+	—	*Br. abortus*
SC	Man	Punjab	—	N/Trans	S	+	+	+	—	+	*Br. melitensis*
NL	Man	Punjab	—	N	R	+	+	+	—	+	*Br. melitensis*
AG	Man	Punjab	—	N	S	+	+	+	+	—	*Br. intermedia*
YPS	Man	Punjab	—	N	S	+	+	+	+	—	*Br. intermedia*
MP	Man	Punjab	—	N	S	+	+	+	+	—	*Br. intermedia*

of no value in the treatment of brucellosis. With the paper-disc method it has been observed that all the strains of *Brucella* were resistant to Sulfonamide compounds, thus corroborating the results of clinical trials. With penicillin only two strains of *Brucella*, one of *Br. abortus* and another of *Br. melitensis*, were sensitive while all the remaining 34 strains were resistant (Table III). Although T'ung (1944) observed that out of 15 strains of *Brucella* tested eight were susceptible to penicillin, most of the other reports indicated that brucellae were resistant to this drug (Spink, 1956). Several reports are available that Streptomycin is both bacteriostatic and bactericidal to brucellae *in vitro* (Hall and Spink, 1947; Yow and Spink, 1948; Santivanez, 1946; Coles, 1948; Kurancowa, 1953). Kurancowa (1953) reported that prolonged cultivation in the presence of this drug led to the development of a drug-resistant strain, which remained resistant through serial passages. In the present study however, it was observed that 15 out of the 36 strains were only slightly sensitive to Streptomycin and 12 of them gave similar reaction to Dihydrostreptomycin, indicating that the strains tested were not so highly sensitive to Streptomycin as compared with the six other antibiotics, to which all strains were highly sensitive. While Yow and Spink (1949) observed that although Chlortetracycline (Aureomycin) did exhibit bacteriostatic activity against brucellae, comparatively its antibrucella action was inferior to that of Streptomycin. Lacy and Lankford (1949), testing 26 strains of *Brucella*, observed that Chlortetracycline is definitely more effective than Streptomycin and that the concentrations of Streptomycin required to inhibit growth were two to five times greater than that of Chlortetracycline. Terni and Tesi (1949) also observed that all the 61 strains of *Brucella* tested in fluid medium were inhibited by 1 mg. per ml. of Chlortetracycline. Spink (1956) observed that Chlortetracycline and Oxytetracycline have the same merit for treatment of brucellosis and that Chloramphenicol (Chloromycetin) does not appear to be as effective as the

TABLE III. VARIABLE REACTION OF DIFFERENT STRAINS OF *BRUCELLA* TO THE TEN CHEMOTHERAPEUTIC AGENTS

Chemotherapeutic agent	Number of strains of *Br. abortus* (total—25)			Number of strains of *Br. melitensis* (total—5)			Number of strains of *Br. suis* (total—3)			Number of strains of *Br. intermedia* (total—3)		
	Highly sensitive	Sensitive	Resistant	Highly sensitive	Sensitive	Resistant	Highly sensitive	Sensitive	Resistant	Highly sensitive	Sensitive	Resistant
Chloramphenicol	19	5	1	3	2	—	3	—	—	3	—	—
Dihydrostreptomycin	14	11	—	5	—	—	2	1	—	3	—	—
Streptomycin	11	14	—	5	—	—	2	1	—	3	—	—
Altafur	25	—	—	4	1	—	3	—	—	—	3	—
Neomycin	13	12	—	5	—	—	3	—	—	2	1	—
Novobiocin	16	9	—	5	—	—	3	—	—	3	—	—
Nitrofurantoin	18	5	2	4	1	—	2	1	—	—	3	—
Aerosporin (Polymysin B)	2	18	5	1	3	1	—	2	1	—	3	—
Ilotycin (Erythromycin)	—	1	24	1	—	4	1	2	—	—	—	3
Penicillin	1	—	24	1	—	4	—	—	3	—	—	3

above two drugs. It was also observed by him that Tetracycline is just as effective as Chlortetracycline or Oxytetracycline. The results obtained in this study with the paper-disc method corroborate these observations. With Chloramphenicol one strain of *Br. abortus* was resistant, while five strains of *Br. abortus* and two strains of *Br. melitensis* were only slightly sensitive to this drug. While most of the reports on chemotherapy for brucellosis were carried out with the classical types of *Br. abortus*, *Br. melitensis* and *Br. suis*, in this study three strains of *Br. intermedia* (Renoux, 1952) were included. These strains resembled in their sensitivity broadly with *Br. melitensis*, except in their reaction to Altafur and Nitrofurantoin. While all strains of *Br. abortus*, *Br. suis* and four out of five strains of *Br. melitensis* were highly susceptible to Altafur, all the strains of *Br. intermedia* were only sensitive to this drug. In the case of Nitrofurantoin also all the strains of *Br. intermedia* were only sensitive, while most of the other strains were highly susceptible to this drug. All the strains of *Br. intermedia* were resistant to Ilotycin and Penicillin. In this study three strains of *Br. abortus* isolated from buffaloes were included, as there was no report so far of any trial of drug sensitivity with such strains. These buffalo strains showed similar characters like those of other bovine strains of *Br. abortus*. Recently, White *et al.* (1963) described a correlation between Penicillin sensitivity and serotypes of coagulase-positive staphylococci isolated from bovine udder, suggesting the possibility of utilising this test for identification of serotypes. The results of drug sensitivity

of these *Brucella* strains did not indicate any such serological relationship with the chemotherapeutic agents tested.

SUMMARY

The sensitivity of 36 strains of *Brucella* isolated from human and animal sources in India including 25 strains of *Br. abortus*, eight of *Br. melitensis*, three of *Br. suis* and three of *Br. intermedia* were tested by paper-disc method against 32 different chemotherapeutic agents. The serological and biochemical characters of each strain were studied prior to the drug-sensitivity test. Except Staphcillin (Methycillin), which was used at one concentration, the remaining drugs were tested at two concentrations. All the 36 strains were highly susceptible to Chlortetracycline, Dimethychlorotetracycline, Tetracycline, Oxytetracycline, Kanamycin and Mandelamine, while they were resistant to 16 of the drugs tested including different Sulfonamide compounds tried. They had variable reaction with ten of the drugs. Except two strains the rest were resistant to Penicillin.

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INVESTIGATION OF AFRICAN HORSE-SICKNESS IN INDIA

II. REACTIONS IN NON-IMMUNE HORSES AFTER VACCINATION WITH THE POLYVALENT AFRICAN HORSE-SICKNESS VACCINE

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OVER a number of years Alexander developed an attenuated polyvalent live virus vaccine against African horse-sickness (AHS). The earliest vaccine introduced in 1934 contained four attenuated strains. As more antigenic types were discovered they were rendered avirulent by serial intracerebral passage in adult mice (Alexander, 1933) and were incorporated in the vaccine. The vaccine in current use consists of seven attenuated strains, and is administered in a single subcutaneous dose. The horses are kept at rest for the following three weeks. The earlier reports of reactions to the vaccine described a febrile response in the majority, systemic reaction in some and oedema of the supraorbital fossa in less than 0·5 per cent of the vaccinated animals (Alexander *et al.*, 1936). The latest information, however, summarizes the vaccine reactions as ' detectable in less than 20 per cent of the vaccinated horses, ' when the horse may be ' off-colour ' for a day or two between seven and nine days after vaccination (Alexander, 1961).

After recognition of AHS in this country (Shah, 1964), vaccine was imported from the Onderstepoort Laboratory in South Africa for the National Horse Breeding Society of India through the good offices of the Rockefeller Foundation and the Ministry of Agriculture, Government of India. The vaccine was received on August 14, 1960, and was administered to horses in the racing stables and stud farms. The present report deals with reactions to the vaccine in two stud farms near Poona, and the laboratory investigations on post-vaccination viremia and antibody response.

MATERIALS AND METHODS

The shipment of 2,500 ampoules was made from 11 lots of vaccine; each ampoule bore the label APSI, AHS 2-2-61 followed by the lot number and contained a single dose of freeze-dried vaccine. Each ampoule was rehydrated individually. No record was maintained of the lot number of the vaccine issued to the different stud farms.

At the Yeravada Stud Farm, situated on the outskirts of Poona, 84 horses (four stallions, 40 brood mares, 16 yearlings, 16 foals, three race horses, one colt, two teaser ponies and two ponies) were vaccinated on August 15, 1960. Temperature records

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†The Virus Research Centre is jointly maintained by the Indian Council of Medical Research and the Rockefeller Foundation.

were taken daily from a day before vaccination to 21 days after vaccination, except on days one, two and five. All horses were observed for clinical reaction.

At the Manjri Stud Farm, located seven miles from Poona, 212 horses (14 stallions, one gelding, 116 brood mares, 28 yearlings, 39 foals, eight ponies and six weanlings) also were vaccinated on August 15, 1960. Daily temperature and clinical records were taken on a group of 35 animals (seven stallions, five brood mares, 12 yearlings, ten foals, and one teaser pony) from the second to the 31st day after vaccination. If severe reactions occurred in animals not in the above 35, they were brought into the observation group.

Techniques of virus isolation, identification, complement fixation and neutralization tests have been described earlier.

RESULTS

At the Yeravada Stud Farm a rise of temperature above 101·5°F was observed for ten animals: five foals, two yearlings, two mares and one stallion. For five of these (one foal, both yearlings, one mare and one stallion) the rise was for a single day, between the fifth and 20th days; for three foals it was for two days, between the fifth and 14th days; and for one foal and one mare for three days, between the sixth and 13th days. The highest recorded temperature was 104·4°F.

Though the febrile reaction was mild, most of the animals were less active and showed signs of general depression. When the animals were let out of their paddocks 15 days after vaccination, they, instead of galloping around, merely cantered listlessly for a short time and then settled down to grazing. This change in behaviour was very marked and affected practically all the animals. They did not regain their usual liveliness till about two to three months after vaccination. Their feed intake was not reduced.

An additional complication was seen in two foals at foot. They developed, on the 37th and the 43rd day after vaccination, incoordination of gait, weakness and fever lasting for three days. Both animals made a complete recovery.

About ten weeks after vaccination an outbreak of abortion occurred on the Farm affecting eight out of a total of 24 pregnant mares in five days. Virus was not isolated from the foetal tissues or from the blood of aborting mares and there was no evidence of any relation between the abortions and vaccination.

At the Manjri Stud Farm the reactions were much more severe. In the 35 animals, with daily records, temperature above 101·5°F was recorded in 22 animals as follows: in all the ten foals, eight out of twelve yearlings, two out of seven stallions, one out of five brood mares and in the single teaser pony. The fever in the brood mare and the teaser pony was confined to a single day on the 12th and 13th days respectively. Of the two febrile stallions, one had fever for a single day (13th day) and the other on the fourth, fifth and 12th to 15th post-vaccination days. The febrile reactions in foals occurred between the fourth and 13th days; fever for more than seven-day duration was seen in seven out of ten foals. In the yearlings fever in eight animals was recorded between the 5th and 23rd days; for a single day (13 and 16) for two, and for three to six days for the remaining six.

In the 35 observed animals swelling of the temporal fossae was noticed between the 12th and 21st days in 17 animals as follows: in seven out of 12 yearlings, two out of seven stallions and one out of five brood mares. Amongst the animals not in the original group for daily observation this sign was seen in two foals and 12 brood mares. The swelling lasted for about a week and was often unaccompanied by fever.

Incoordination of gait was noticed in three yearlings and three foals (four and a half to six months old). The onset in the yearlings was 31, 37 and 42 days, and in foals 17, 19 and 30 days after vaccination[1].

The incoordination became most marked three to five days after onset, after which gradual but complete recovery began. The animals were febrile for the first two to three days and were dull, drowsy, dazed and off-feed.

A single death occurred on the Farm in the one month following vaccination. The fatal case was a 13-year-old mare, which was in a debilitated state at the time of vaccination. After vaccination she went rapidly downhill, developed swelling of the temporal fossae on the 13th day and died on the 14th day. At autopsy the pericardial sac contained a large quantity of fluid; and the lungs were oedematous. A strain of AHS vaccine virus was isolated from the spleen of this animal.

Sixteen abortions occurred in 63 pregnant mares between August 17, 1960 and April 7, 1961. This rate of 25 per cent was higher than the average annual rate of 10 to 15 per cent on this Farm. The episodes of abortion were scattered over nine months and it is not known if they had any direct or indirect relation to vaccination.

Post-vaccination viremia: At the time of vaccination the study of post-vaccination viremia was not visualized. As severe reactions occurred in the horses of the Manjri Stud Farm, heparinised blood samples were collected from these animals. Later, specimens were obtained from other localities from horses with mild or severe reactions. The clinical status of the horses at the time of bleeding and results of virus isolations (after intracerebral inoculation of mice) are given in Table I. It is seen that a large proportion of the animals examined for viremia were reacting severely.

TABLE I. FREQUENCY OF VIRUS ISOLATION FROM HEPARINISED BLOOD OF VACCINATED HORSES

Days after vaccination	Locality	Clinical status	Number tested	Positive	Negative	Positive log. of LD_{50} titre per 0·30 ml.
14	Manjri Stud Farm, Poona	Severe reaction	5	4	1	1·7, 2·1, 1·2, 2·3
16	Gwalior Stables, Poona	Severe reaction	1	1	0	1·5
19	Manjri Stud Farm, Poona	Severe reaction	2	1	1	3·0
23	Haffkine Institute, Bombay	Severe reaction	6	4	2	Not done
23	Haffkine Institute, Bombay	No reaction	5	2	3	Not done
42–43	Poona	Febrile	2	0	2	
49	Gwalior Stables, Poona	Severe reaction	8	1	7	0·5
71*	Haffkine Institute, Bombay	Normal	6	0	6	
85	Yeravada Stud Farm, Poona	Aborting mares	8	0	8	
			43	13	30	

*2nd bleedings on six horses which were viremic on day 23.

[1]There were seven mules on the Farm and one of them showed fever, incoordination of gait and ataxia on the 28th day after vaccination.

Virus strains were isolated from six out of eight specimens from day 14 to 19 post-vaccination, six out of 11 of day 23, none out of two of day 42 to 43, and one out of eight of day 49. The six horses of the Haffkine Institute, viremic on day 23, were re-bled on day 71 and the blood samples were tested for virus. No virus was isolated. Also, virus was not detected in specimens collected 85 days after vaccination from the eight aborting mares at Yeravada Stud Farm, within three to seven days of their abortions. The strains were identified as AHS by quick complement fixation tests. Quantitative virus estimations for seven specimens gave titres ranging from $10^{0.5}$ to $10^{3.0}$ $LD_{50}/0{\cdot}03$ ml. of blood.

The pattern of illness and mortality in adult mice inoculated with blood from vaccinated horses was quite different from that in mice inoculated with blood of naturally infected animals. The incubation period of strains from vaccinated horses was four to six days and death occurred within a day or two of illness. The onset of illness in all mice of a group was simultaneous or over a one- to two-day-period, and recovery from sickness did not occur.

TABLE II. MORTALITY AND MORBIDITY PATTERN IN ADULT MICE INOCULATED INTRACEREBRALLY WITH HEPARINISED BLOOD FROM (A) SICK HORSES FROM JAIPUR EPIZOOTIC AND (B) VACCINTED HORSES

Days after Inoculation	(A) Jaipur strains									(B) Strains from vaccinated horses								
	603389-1 (>0·5)*			603344 (>0·5)			603395 (>0·5)			605286 (1·7)			605287 (2·1)			605288 (1·2)		
	D	S	P	D	S	P	D	S	P	D	S	P	D	S	P	D	S	P
1																		
2																		
3																		
4													2	1	1			
5										5		1	3	2		1	3	1
6										1	1		2			4	2	
7										1						2		
8																		
9																		
10																		
11									1									
12																		
13								2	2									
14								2										
15								4										
16							1	3										
17						3		3										
18			3		1	2		3										
19		1	3			5		3										
20			3			5	1	2										
21			4		2	2	1	2										
22			4	1		2		2										
23			6			2	1	1										
24			6					1										
25		1	5					1										
26			5					1										

*Figures in parenthesis are positive log. of LD_{50} titres in heparinised blood. For the Jaipur strains titres calculated on morbidity ratios and for strains from vaccinated horses, the morbidity ratio was identical with mortality ratio.

D=dead; S=sick; P=passed.

In Table II the first passage mortality and morbidity of three strains from vaccinated horses are compared with those of three epizootic strains. The differences suggest that the former are mouse-adapted viruses.

At the time of collection of positive blood specimens from vaccinated horses natural disease was not present in these localities, and it was presumed that the isolated viruses were one or more of the attenuated vaccine strains. The nature of illness in mice supported this assumption. Further, six strains from vaccinated horses (one from the spleen of the fatal case and five from heparinised samples of blood) were screened against two convalescent horse sera from Jaipur. The antibody titres of these sera against seven epizootic strains from three States ranged from 1/22 to 1/168 (Shah 1964). None of the six strains from vaccinated horses were neutralized by either of these sera in 1/10 dilution, an indication that they were unlike the Indian epizootic virus.

Investigation of the possibility of transmission of vaccine viruses

On the Manjri Stud Farm, *Culicoides* were present in good numbers and, as shown above, the horses circulated the vaccine viruses for a considerable length of time. A limited attempt was, therefore, made to see if field evidence for transmission of vaccine viruses could be found.

On November 2, 1960, about ten weeks after vaccination, serum specimens were obtained from 11 healthy unvaccinated horses. Six of them were on the Farm or in close proximity and the remaining five within one mile distance. Complement fixing or neutralizing antibodies were not detected in these sera. Further, three strains isolated from unvaccinated sick animals in Poona were identified as similar to other epizootic strains and unlike the vaccine strains.

Antibody response to the vaccine: Thirty-two of the Manjri Stud Farm horses and 35 of the Yeravada Stud Farm horses were bled at five and seven weeks, respectively, after vaccination. Second bleedings were made of 32 out of the 35 horses on Yeravada Stud Farm, 18 weeks after vaccination.

The complement fixing antibody titres of these sera against Jaipur strain 60,3395 are summarized in Table III. CF antibodies were demonstrable in all but one of the 67 horses bled at five to seven weeks, and titres were generally higher in the group bled at five weeks. At 18 weeks the titres were lower than at seven weeks and CF antibodies were not detectable or barely detectable in 12 out of the 32 specimens. The mean geometric titres at five, seven and 18 weeks after vaccination were 1/128, 1/28 and 1/8, respectively. It should be noted that while the sera collected 7 and 18 weeks post-vaccination were from the same group of animals at the Yeravada Stud Farm, those of five weeks post-vaccination were from a different, more severely reacting group at the Manjri Stud Farm.

The neutralizing antibody titres of the 67 sera (of five and seven weeks) against the Indian strain are given in Table IV. With virus doses as indicated in the Table neutralizing antibodies were not detected in 32 out of the 67 sera in the lowest dilution of 1/10. In 25 the titre was less than 1/10, but the average survival time (AST) was significantly prolonged at 1/10 dilution, indicating partial protection. Six had titres between 1/10 and 1/50 and one $>1/50$. The titres of the two control immune sera

(convalescent sera from naturally infected Jaipur horses) varied from 1/82 to 1/834 for one and 1/62 to 1/250 for the other.

TABLE III. TITRES OF COMPLEMENT FIXING ANTIBODIES IN SERA OF VACCINATED HORSES TO ANTIGEN FROM THE INDIAN STRAIN

Locality	No.	Weeks Post-vaccination	Titres									Mean geometric titre
			<1/4	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	
Manjri Stud Farm	32	5	0	0	0	2	2	7	10	5	6	1/128
Yeravada Stud Farm*	35	7	1	1	2	8	11	12				1/28
Yeravada Stud Farm ..	32	18	6	6	10	3	5	1	1†			1/8

*Samples of 32 of above 35.
†Titre >1/64. Probably infected by Indian strain.

TABLE IV. ANTIBODY TITRES OF SERA OF VACCINATED HORSES AGAINST JAIPUR STRAIN 603400

	Run	Dose Log. LD_{50}	Titre of immune control		No Protection	Titre <1/10 but AST+at 1/10	1/10 to 1/50	Over 1/50
			603369	603339-4				
A. 32 horses (5 weeks)	805	3·0	1/146	1/70	8	4	1	0
	806	2·4	1/834	1/250	4	8	1	0
	810	3·1	1/780	1/146	4	1	1	0
	Sub-total				16	13	3	0
B. 35 horses (7 weeks)	809	3·5	1/82	1/62	10	4	0	0
	810	3·1	1/780	1/146	4	1	1	1
	811	2·5	>1/50	≯1/50	2	7	5	0
	Sub-total				16	12	6	1
Total 67 horses					32	25	9	1

The amounts of virus in some of the above tests were high; for this reason the sera examined in tests 809 and 810 were re-checked in two tests with 1·8 and 2·3 log LD_{50} of virus. Serum was tested in five five-fold dilutions beginning at 1/2. In each test seven convalescent sera from naturally immune animals collected between 32 and 47 days after onset were included for comparison. The titres of the sera from 27 vaccinted horses were <1/10 for 21 and between 1/10 and 1/50 for six. In the 21 sera with titre <1/10 the results at serum dilution 1/2 were as follows: four protective,

ten partially protective and seven negative. Titres of the 14 convalescent sera from naturally infected horses ranged from 1/250 to >1/1250.

Alexander (1935) reported that maximum neutralizing antibody response may sometimes be delayed for as long as three to six months after vaccination. Twenty-four pairs of sera of 7 and 18 weeks post-vaccination were, therefore, examined against the Indian strain in serum dilution neutralization tests. Both members of a pair were included in the same test. The virus dose varied from 1·8 to 2·6 log. LD_{50}. Greater than four-fold increase in antibody titre in the 18-week specimens was detected for 5 out of 24 pairs. In the remaining 19 pairs antibody titres in the 7- and 18-week specimens were not significantly different. As epizootic AHS had appeared at Poona about six to seven weeks after vaccination, it was not possible to decide if the rise in antibodies was a result of infection by the epizootic virus or a late effect of the vaccine.

DISCUSSION

The reactions to the vaccine in the horses at Yeravada and Manjri Stud Farms differed in many important respects from those described by the South African workers. Activity was depressed in most animals for 6 to 12 weeks after vaccination. A number of horses at the Manjri Stud Farm were very ill and the symptomatology closely resembled mild or moderately severe natural disease. The febrile reactions were most marked in foals and yearlings. It is possible that the vaccine contributed to the death of one mare. Vaccine from the same shipment was administered to the horses on both farms and there was no apparent explanation for the wide difference observed in the severity of reaction. It was not possible to ascertain if the severe reactions were confined to animals given any one lot of the vaccine.

The most noteworthy finding in the vaccine reactions was the mild and transient neurological involvement seen in foals and yearlings, three to seven weeks after vaccination. Affection of the central nervous system (CNS) is not described in natural AHS and this neurotropic propensity of the vaccine may well be the result of prolonged propagation in mouse brain. Alexander *et al.* (1936) described a somewhat similar picture in a horse given one of the AHS strains intracerebrally. To establish conclusively the aetiologic relationship of the CNS symptoms to the vaccine and to determine if any one strain is responsible for the effects, it would be essential to carry out histo-pathological and virological investigations of CNS and other tissues of animals sacrificed or dead at the time of occurrence of CNS symptoms. This could not be done in the present study. Encephalitis after vaccination has been observed in donkeys in Cyprus (Reid, 1960). Alexander (1961) recently reported that one strain of vaccine virus (Karen Group 7) was capable of breaking the blood-brain barrier in nearly all guinea-pigs injected intraperitoneally, with the result that they developed fatal encephalitis. This enhanced neurotropism was not shown by any of the six remaining vaccine virus strains.

Alexander (1936) reported that viremia after vaccination is transient and low titred, and is more likely to be associated with a severe reaction. In this study virus was present in blood with a high frequency and in moderate titres between 14 and 23

days after vaccination. The single positive in eight specimens 49 days after vaccination may represent a chance finding of an uncommon occurrence. This long duration of viremia in vaccinated horses is in contrast with the shorter period in natural disease, and may be related to the fact that seven live viruses are simultaneously introduced by vaccination. By arrangement with the Director of Veterinary Services, Republic of South Africa, the strains of virus isolated from the vaccinated horses were forwarded to Onderstepoort for identification as to their types. While this study is not yet complete, it has been evident that at least some of the isolates are mixtures of two and probably more virus strains and that no one single type is responsible for the demonstrated viremia (Personal communication).

It is of importance to note that the group of animals studied for viremia was not chosen by an unbiased random selection from all vaccinated horses, but had a large proportion of severely reacting horses. It is possible that the viremia pattern in non-reacting animals may be different from the one described.

The investigation of possible transmission of vaccine viruses did not reveal evidence in favour of such transmission. However, the number of unvaccinated horses examined was very small. It would seem worthwhile to test this possiblity by immunization of only a proportion of the animals in an area where: (a) there is a large horse population, (b) *Culicoides* are present, and (c) vaccination is desirable but not urgent.

Complement fixing antibodies declined to low levels by the 18th week. Neutralizing antibody titres of sera of vaccinated horses were disappointingly low as measured against the Indian virus and compared unfavourably with the high titres of sera from naturally infected animals. This was not surprising, as the Indian virus in its antigenic make-up is known to be considerably different from the viruses incorporated in the vaccine.

In the absence of a comparable population of unvaccinated horses it was not possible to make a firm estimate of the effectiveness of the vaccine in the field. However, when epizootic AHS appeared in Poona early in October, 1960, about six to seven weeks after vaccination, no case of AHS was observed in over 200 horses in this study or in an additional 600 vaccinated horses in other stud farms and racing stables. In contrast, 35 fatalities due to AHS were recorded in an estimated horse population of 832 in Poona city. The mortality in Poona district was reported as 456 in an estimated population of 2,836 horses. These figures confirm the general impression of a high degree of effectiveness of the vaccine.

SUMMARY

Clinical reactions to the polyvalent African horse-sickness vaccine were studied in two stud farms. Fever and swelling of the temporal fossae were observed in a proportion of the animals. A few foals and yearlings manifested signs of mild transient CNS involvement three to seven weeks after vaccination. The severity of the reaction was very different on the two stud farms, though both received vaccine from the same lot.

Presence of vaccine viruses was detected in 12 out of 19 heparinised blood specimens collected between 14 and 23 days and in one out of eight on the 49th day after vaccination.

Complement-fixing antibodies were detected in 66 out of 67 horses five to seven weeks after vaccination. The titres of neutralizing antibodies in sera of vaccinated horses to the Indian virus were very low in comparison with titres in naturally infected animals.

ACKNOWLEDGEMENTS

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STUDIES ON INDIAN BOVINE BLOOD-GROUPS

I. BUFFALO BLOOD ANTIGENIC FACTORS DETECTED THROUGH CATTLE BLOOD-GROUP REAGENTS

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FOLLOWING the epoch-making discovery of 'blood-groups' in man by Landsteiner in 1901, like work was taken up with different breeds and species of farm animals from which considerable information has become available on their incidence, frequency and mode of inheritance. Irwin (1956) and Rendel (1957) reviewed the work. In cattle as many as 50 different blood-group factors have been discovered; these are controlled by 11 or probably 12 loci (Rendel, 1958). Stormont *et al.* (1951) showed that 21 out of the 38 antigenic factors studied were governed by multiple alleles at one locus—the B-locus. Positive correlations have been reported between some of the blood-group factors and percentage of butter fat (Nair, 1956; Rendel, 1959).

Blood grouping of Indian buffaloes was first attempted by Singh (1942). He tested blood from 48 buffaloes and 159 cattle by cross-matching agglutination test and observed that isohaemagglutinins in the sera and agglutinable factors on erythrocytes were present in both the species. Reda (1957) tested 62 Egyptian buffalo blood samples with human blood grouping reagents A and B and classified them into A, B, AB and O groups. He concluded that buffalo and human agglutinogens were related. Rao and Sadri (1960) studied the antigenic composition of buffalo semen and found that some antigens of buffalo blood serum were also common to the various fractions of semen. In the present paper are reported the results of attempts to detect blood factors, if any, in the Murrah breed of buffalo using cattle blood-group reagents.

MATERIAL AND METHODS

Blood samples were taken from 150 buffaloes and tested with 35 cattle blood-group reagents stocked in the laboratory. All the animals belonged to the Murrah herd of the Institute. Blood samples were collected in isotonic citrate solution from the jugular vein and the red blood cells were separated and washed three times in normal saline before preparing 2·5 per cent suspensions. Out of the 35 cattle blood-typing reagents used in the tests, 28 were prepared by iso-immunization of cattle, five by hetero-immunization of sheep with cattle blood, one (anti-J) from normal sera from cattle and buffaloes and one from normal buffalo serum. Since the reactions of several antibody fractions of unitary character could not be fitted in with those of the reagents described by European and American workers, these reagents were marked as Izatnagar$_1$ (Iz_1), Iz_2 to Iz_{21} in the order of their preparation. The Iz reagents were tested on animals whose blood was typed previously at the Copenhagen blood-grouping

laboratory with the co-operation of Dr. Moustgaard and were found to be different.

On examining the normal sera from buffaloes with cattle red blood cells it was found that out of 75 sera examined 29 reacted with one or more type of red cells. Some of these reacting normal to buffalo sera were fractionated by absorbing with cattle red blood cells and two reagents were prepared from them.

For the detection of antigenic factors haemolytic tests were used. At an early stage the plastic cavity blocks used in haemagglutination tests were found to be more useful for these tests also, as compared with agglutination tubes, and have been used for this purpose in this laboratory since its inception in 1955. With these blocks we have found it comparatively easier to set up the test, easier to shake the whole thing together and more convenient and less time consuming for reading the results. To facilitate the reading of results further, the block or the plate was fixed in a wooden frame with a reflecting mirror below, which could be tilted to the desired angle to suit the convenience of the operator. Each block contains 80 cavities, all of which can be examined at one glance. The only drawback in their use is that during summer months with low humidity (April to June/July) there is rapid evaporation of the fluid and the cavities are sometimes completely dry even before the time the results are due to be read. To overcome this difficulty, all the blocks were placed one upon another after setting up the test and then wrapped in a folded piece of wet cloth, taking care to avoid spilling of the fluid from the cavities during the process of wrapping and shaking.

For setting up the test, two drops of the reagent were placed in the cavity, followed by a drop of 2·5 per cent suspension of thrice washed red blood cells and the two were mixed. After about 10–15 minutes one drop of rabbit serum, fresh or preserved in the deep-freeze, was added as complement and mixed thoroughly. The results were noted after two, four and 16–20 hours for any delayed reaction. Control tests of serum and cells, cells and saline solution, and cells and complement were also set up side by side. Haemolysis of approximately half or more of the red blood cells was taken as positive.

RESULTS

The results of the haemolytic tests of 150 Murrah buffaloes are given in Table I.

TABLE I. OCCURRENCE OF CERTAIN ANTIGENIC FACTORS IN 150 MURRAH BUFFALOES

Antigenic factors	No. of animals possessing the antigenic factor	Fraction of the total	Antigenic factors	No. of animals possessing the antigenic factor	Fraction of the total
Iz_2 ..	65	0·4333	E_1 ..	9	0·0600
Iz_3 ..	98	0·6533	Q ..	18	0·1200
Ix_7 ..	70	0·4666	G ..	19	0·1266
Iz_8 ..	121	0·8066	K ..	3	0·0200
Iz_{13} ..	1	0·0066	J ..	53	0·3533
Iz_{14} ..	17	0·1133	L ..	16	0·1066
Iz_{17} ..	7	0·0466	Z_1 ..	36	0·2400
A ..	77	0·5133	U_1 ..	14	0·0933

Out of the 35 cattle blood-group reagents used in the test, only 16 reacted positively with buffalo blood. Blood from none of the buffaloes reacted with the remaining 19 reagents.

The antigenic factors detected in buffalo blood occurred in varying proportions, the highest being 0·8066 (Iz_8) and the lowest only 0·0066 (Iz_{13}) (Table I). Six factors were fairly common, occurring in more than 35 per cent animals; five others were present in less than 10 per cent of the animals.

In Table II are shown the comparative distributions of the 35 antigenic factors in 150 Murrah buffaloes and 150 Hariana cattle. Out of the 16 antigenic factors present in buffalo blood, all except three occurred much less frequently in buffaloes than in cattle.

TABLE II. COMPARISON OF THE ANTIGENIC FACTORS IN 150 HARIANA CATTLE AND 150 MURRAH BUFFALOES

Antigenic factor	No. of Hariana animals positive for the antigen	No. of Murrah animals positive for the antigen	χ^2	Antigenic factor	No. of Hariana animals positive for the antigen	No. of Murrah animals positive for the antigen	χ^2
Iz_1	2	..	..	Iz_{19}	66	..	..
Iz_2	99	65	15·548**	Iz_{21}	7	..	..
Iz_3	60	98	19·308**	BF_2	10	..	..
Iz_4	73	..	..	A	96	77	4·929*
Iz_5	40	..	..	E_1	62	9	92·007**
Iz_6	84	..	..	Q	134	18	189·520**
Iz_7	112	70	24·641**	G	100	19	91·383**
Iz_8	75	121	31·142**	K	63	3	67·610**
Iz_9	44	..	..	W	71	..	..
Iz_{10}	40	..	..	R	16	..	..
Iz_{11}	46	..	..	X_1	140	..	..
Iz_{12}	23	..	..	V	70	..	..
Iz_{13}	19	1	14·450**	J	50	53	1·333
Iz_{14}	112	17	122·739**	L	133	16	182·520**
Iz_{15}	92	..	..	M	77	..	..
Iz_{16}	1	..	..	Z^1	56	36	6·270*
Iz_{17}	63	7	58·434**	U_1	87	14	79·540**
Iz_{18}	27	..	..				

*Significant at 5%. **Significant at 1%.

NOTE: Where the frequencies were less than 5, Yates correction factor was used.

From a comparative study of the frequency of occurrence of these 16 antigenic factors common to both cattle and buffaloes (Table II), it would be seen that in all cases except one the difference was significant or highly significant. Three antigenic factors, Vz. Iz_3, Iz_8 and J, were found to occur more frequently in buffaloes than in cattle and in two of these cases the differences were highly significant. The case of J antigen, however, was totally different. It was noted with interest that this antigen was found to be present in both the species in an almost equal frequency. Anti-J, which occurred normally, had been isolated from normal sera of cattle as well as buffaloes.

In spite of the differences in the frequency of these antigenic factors in cattle and buffaloes their occurrence in both the species was not without interest. This was particularly so because there were not many points of similarity between the two species and efforts to cross-breed them had proved unsuccessful.

Buffalo normal sera as cattle reagents: Normal sera from 75 buffaloes of Murrah breed were tested with washed red blood cells from 25 Hariana cattle for haemolysis, using fresh rabbit serum as complement. Twenty-nine sera proved positive for antibodies and caused haemolysis of one or more types of cattle red cells.

Out of the 29 positive sera 11 sera which had proved most reactive were collected and were analysed by absorbing and testing them with red blood cells from 25 cattle. By further fractionation and tests two reagents were prepared from these normal sera. One of these behaved exactly like anti-J of cattle and was identical or very similar to the reagent prepared from normal cattle serum. The other reagent, BF_2, was quite different from all the other reagents prepared so far. Two other reagents obtained from normal sera of buffaloes at first appeared to be different from others. But by further tests it was found that they were also similar to anti-J and that certain discrepancies in reactions observed were due to inhibitory action of the pre-zone phenomenon which had been noticed more frequently with buffalo sera than with cattle sera.

SUMMARY

Blood samples from 150 Murrah buffaloes were tested with 35 cattle blood-group reagents and the results were compared with those of 150 Hariana. Out of 35 antigenic factors, 16 appeared to be common to the two species, though the frequency varied significantly, except in case of J factor.

Two reagents for blood-grouping of cattle were prepared from buffalo normal sera.

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EFFECT OF ENVIRONMENTAL FACTORS ON WOOL GROWTH

I. EFFECT OF NORMAL CLIMATIC FACTORS

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A NUMBER of workers have reported that environmental factors affect wool production (Miller, 1933; McMahon, 1940, 1942; Wildman, 1944; Carter, 1951; Doepel and Turner, 1959). One of these factors is the climatic stress resulting from the operation of air temperature, humidity, air movements, solar radiation and barometric pressure, acting singly or in combination (Lee and Phillips, 1948).

The positive effect of higher temperatures on increase in wool production has been described by Thompson (1953), W.I.R.A. (1957), Wildman (1958) and Graham *et al.* (1959). Findlay (1954), Findlay and Beakley (1954), Fergusson (cf. F.A.O., 1955) and Maule (1956), however, stated that rise in temperature adversely affected the wool production. Marston (1935) and Coop (1953) reported that temperature did not influence the growth rate of wool. Humidity has been considered by many authors in conjunction with rainfall. Thus, Nanda (1947) reported that Merino sheep could not survive in Uttar Pradesh because of high rainfall and heavy humidity. Lee (1950) and Findlay (1955) reported that humidity had a marked effect only at high temperatures. Knapp and Robinson (1954) found that sheep could withstand high temperature but not high humidity. Photoperiodicity, i.e., the duration of day length, has been found to affect the wool production (Coop, 1953; Yeates, 1954; Symington, 1959). No systematic study on the effect of climatic factors on wool production has been carried out in India.

In this study experiments carried out on Bikaneri sheep are described. The factors considered were air temperature, vapour pressure (humidity), diurnal varying day length and maximum air temperature.

MATERIAL AND METHODS

Thirty-six ewes of the Chokla type of Bikaneri sheep, above one year in age, from the flock maintained at the Institute, were selected for the experiment conducted during the period March 1, 1959 to March 14, 1960. The ewes were kept without breeding during the experiment and a year prior to it to eliminate all possible effects of gestation, lactation, etc., on the wool production. The animals were shorn off their fleece in the middle of February, 1959, and were washed twice with soap and running

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water. The animals were weighed consecutively on three days at the end of February, and on the basis of the average live-weights their nutritive requirements were calculated according to Morrison's Feeding Standards for sheep; the requirements were revised monthly on obtaining the monthly live-weights of the ewes. The ewes were stall-fed and their nutritive requirements were adequately met with concentrate mixture and fodder. Concentrate mixture composed of 30 parts of groundnut cake, 25 parts of crushed gram, 25 parts of crushed barley and 20 parts of wheat bran making the D.C.P. constituents of the ration as 19·54 per cent and T.D.N. constituents as 72·58 per cent. In addition, common salt and churn brand I.C.I. mineral mixture, each 5 gm./head/day, were added to the total concentrate mixture. The feeding was done in galvanised iron troughs in a group lot. Fodder consisted of wheat *bhoosa* fed *ad lib.* and 2 lb. of green tree leaf fodder (*pakker* leaves—*Ficus infectoria*)/ head/day. Water was kept in galvanised iron troughs and made accessible to animals at all times. The sheds were cleaned twice a day. The feeding was done between 7·30 A.M. and 8·30 A.M. and 1·30 P.M. and 2·30 P.M. every day. The animals were not allowed to pasture during the experiment; their vitamins A and D requirement was met with by feeding 'Trivax' Veterinary Vitamin Oil (Kerala Government Factory, Kozikode, Kerala) with potency of 6,000 i.u./gm. of vitamin A and 600 i.u./gm. of vitamin D in doses as recommended by Nutritional Research Committee, U.S.A. (Mattson, 1954; Jones, 1954). This was drenched twice a week on Wednesdays and Saturdays to individual animals.

The wool production was determined in six regions from 4 sq. cm. areas tattooed on the left side of the animal. These regions (Fig. 1) are described below.

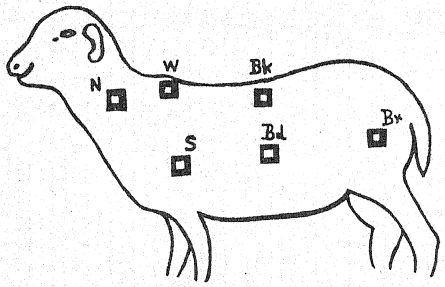

FIG. 1. DEMARCATED REGIONS FOR THE ESTIMATION OF WOOL PRODUCTION

W = Withers S = Shoulder
Bk = Back Bd = Body
N = Neck Br = Breech

Withers .. At the highest point of scapula on the supraspinous fossa about half an inch below the mid-dorsal line.

Back .. About half an inch below the mid-dorsal line on the 12th thoracic vertebra.

Neck .. On the 4th cervical vertebra.

Shoulder .. On the scapula about one inch above the head of humerus towards the right side.

Body .. At the junction of the 11th rib with the costal cartilage in line below the back region.

Breech .. On the femur about one inch above the patella. The upper demarcating line of each tattoo mark was parallel to the mid-dorsal line. The sampling of the wool was done at an interval of every 30 days. The bone dry weights of the samples were determined with the help of a torsion balance as per the method described by Ramachandran and Yeri (1954).

The wool production of the animals was recorded for three periods of 30 days each before grouping them for further studies. But due to an accidental fire in the laboratory part of the samples of the third period were lost. Hence, the wool production of the first two periods, for 30·4 days each ($365 \div 12$), was calculated for each animal and for each region. From this the average wool production per sq. cm. of body area for 30·4 days for each animal was calculated. The mean wool production from the average of the two periods (Standardization period) was then found out (Table I) and on the basis of these data the animals were grouped into trios (blocks).

TABLE I. EXPERIMENTAL EWES AND THEIR WOOL PRODUCTION IN MG. (PER CM.^{2} OF BODY AREA FOR 30·4 DAYS) DURING THE STANDARDIZATION PERIOD

Trio (Block) No.	Control group		L_1 group		L_2 group	
	Tattoo No. of ewe	Wool production (mg.)	Tattoo No. of ewe	Wool production (mg.)	Tattoo No. of ewe	Wool production (mg.)
1	215	15·29	98	15·14	232	15·26
2	102	15·86	203	15·81	112	15·99
3	117	16·37	185	16·09	214	16·59
4	141	17·95	205	17·48	119	17·60
5	231	19·60	121	19·99	235	19·29
6	152	18·91	128	18·61	260	18·12
7	248	14·87	201	14·78	187	14·84
8	200	14·50	273	17·86	217	16·68
9	170	13·15	166	13·62	190	14·24
10	110	14·58	78	9·74	188	11·63
11	84	9·43	45	10·78	233	10·06
12	249	11·42	94	12·12	88	11·72
Average wool production of each group		15·16		15·17		15·17
Average wool production of each group eliminating trios 11 and 12		16·18		15·91		16·02

One animal from each of the trios was alloted to each one of the three groups, L_1, L_2 and control (Nagarcenkar and Bhattacharya, 1964); the groups were formed on the basis of similar average wool production. The normal diurnal day light duration was calculated with the use of tables in the Indian Ephemeris and Nautical Almanac for the year 1959. The time of sunrise and sunset was obtained by interpolations for the longitude and latitude of Izatnagar (Long. 79° 25'E and Lat. 28° 20'N). The actual duration of day light (Fig. 2) was calculated as per the methods of Yeates (1954). Data on the atmospheric temperature and humidity were maintained with the help

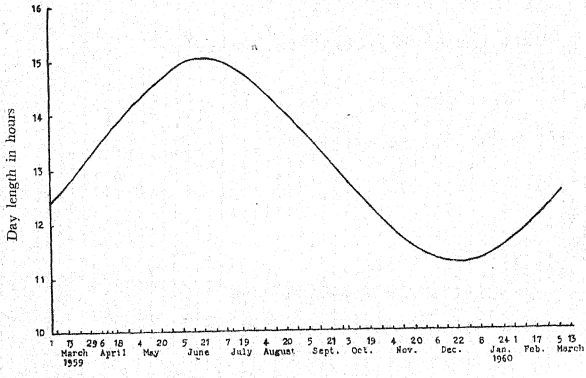

FIG. 2. DAY LENGTH DURATION AT IZATNAGAR (LAT. 28° 20'N LONG. 79° 25'E) THROUGHOUT THE YEAR

of a hand psychrometer four times a day at 6 A.M., 10 A.M., 2 P.M. and 6 P.M. The minimum and maximum temperatures were recorded once in 24 hours at 6 A.M. daily. The animals were shorn during autumn in three instalments in August, September and October and during spring in March immediately on completion of the experiment. During the course of the experiment one ewe (No. 84) from the Control group died. Another ewe (No. 249) from this group was sick for a long time and had much reduced vigour. The results are, therefore, based on the rest of the animals. The ewes of Control group were kept in a pen 16·5 ft. × 14 ft. with thatched roof, split bamboo walls and *murum* flooring. The walls of the shed on two sides were covered with jute curtains to prevent direct sunlight in the paddocks. Feeding and watering arrangements were done inside the pen.

RESULTS

The data on the effect of normal climatic factors on the ten ewes belonging to the control group have been presented below and graphically represented in Fig. 3.

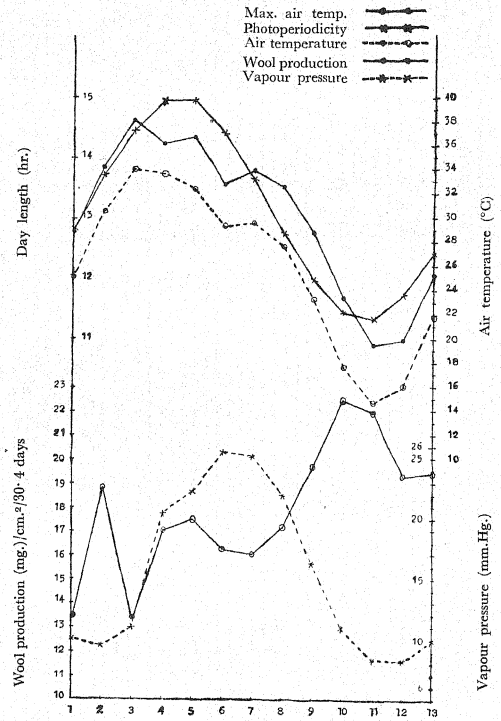

FIG. 3. WOOL PRODUCTION AND CLIMATIC CONDITIONS THROUGHOUT THE YEAR

Relationship between air temperature (°C) and wool production (mg./cm.²/30·4 days): The daily mean air temperature and the average periodic Air temperature for each of the 13 periods from March 1, 1959 to March 14, 1960 were worked out from the data on dry bulb temperature (°C) maintained with the help of a hand psychrometer four times a day. The data were analysed statistically, and the correlation coefficient between Air temperature and average Wool production was found to be –0·713, which is highly significant ($P < 0{\cdot}01$). This indicates that atmopheric temperature affects

wool production. With high air temperature the wool production is hampered, and with the fall in air temperature there is an increase in wool production (Fig. 4).

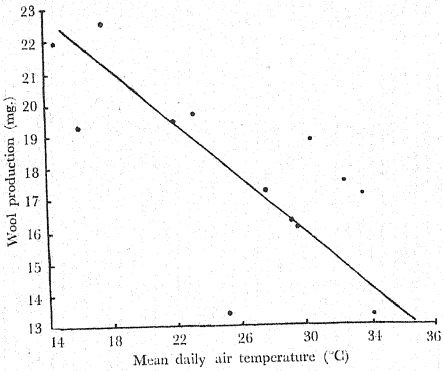

FIG. 4. RELATION BETWEEN WOOL PRODUCTION AND AIR TEMPERATURE

Relationship between vapour pressure (mm. Hg.) and wool production (mg./cm.²/30·4 days): The vapour pressure was based on the data on dry and wet bulb temperatures maintained with a hand psychrometer at 6 A.M., 10 A.M., 2 P.M. and 6.P.M. From this the daily mean vapour pressure was calculated and then the average periodic vapour pressure for each of the 13 periods was taken.

The correlation coefficient between the vapour pressure and the average wool production was found to be -0·324, which is not significant statistically. Thus, it is seen that the vapour pressure, i.e., atmospheric humidity has no significant effect on the production of wool in the Bikaneri ewes. This is also clear from Fig. 3, wherein an uniform trend of relationship of wool production with vapour pressure is not revealed as in case of other climatic factors.

Relationship between diurnal photoperiod (day length in hours) and wool production (mg./cm.²/30·4 days): The correlation coefficient between the mean periodic diurnal photoperiod and the average wool production was calculated. It was found to be -0·667 which is statistically significant ($P<0·05$). This indicates that, the wool growth in Bikaneri sheep was linearly related with the day length and with a decreasing photoperiod there was an increase in wool growth and *vice versa* (Fig. 5).

Relationship between maximum air temperature (°C) and wool production (mg./cm.²/30·4 days): From the data on the maximum air temperature (°C) maintained daily, the mean maximum air temperature for each period was calculated. On statistical analysis of the data, the correlation coefficient between the maximum air temperature and the average wool production was found to be—0·687, which was highly significant

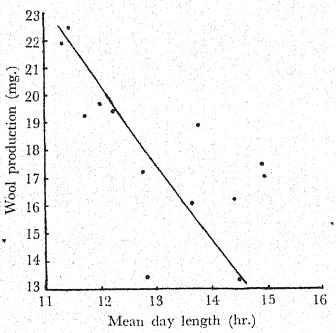

FIG. 5. RELATION BETWEEN WOOL PRODUCTION AND DAY LENGTH (PHOTOPERIOD)

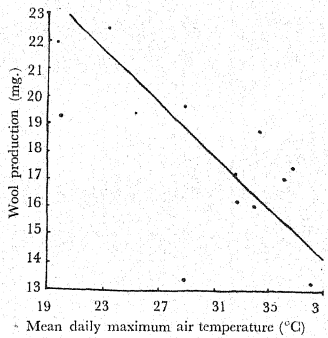

FIG. 6. RELATION BETWEEN WOOL PRODUCTION AND MAXIMUM AIR TEMPERATURE

($P<0{\cdot}01$). This indicates that the wool growth is linearly and negatively related with maximum daily air temperature (Fig. 6), and as in the case of the mean daily air temperature, the wool production decreases with increase in daily maximum air temperature and *vice versa*.

Partial correlation and multiple correlation between wool production and climatic factors: As the air temperature and photoperiod of the three main climatic factors, viz., temperature, humidity and day length, control the wool growth, the partial correlation between both of these climatic factors independently and wool production were calculated, keeping the other factor constant. The correlation coefficient between air temperature and photoperiod (day length) was first calculated and found to be 0·944, which was highly significant ($P<0{\cdot}001$). The partial correlation coefficient between wool production and air temperature, keeping photoperiod constant, was found to be –0·339, which was not significant statistically. Similarly, the partial correlation coefficient between wool production and photoperiod, keeping air temperature constant, was found to be 0·026, which was also not significant statistically. Thus, air temperature and photoperiod were showing effects only in the presence of each other and were not independently affecting wool production. As such, multiple correlation was further undertaken. The values of partial regression coefficients, multiple correlation coefficient and multiple regression equation are given in Table II. The partial regression values were seen to be not significant and multiple correlation significant at 5 per cent level. It is, therefore, conclusively proved that both air temperature and photoperiod jointly influence the wool production.

DISCUSSION

Of the climatic factors examined, only the air temperature and photoperiod affected wool production; humidity did not significantly affect the wool growth in

TABLE II. PARTIAL REGRESSION AND MULTIPLE CORRELATION VALUES

Wool production × Air temperature, keeping photoperiod constant	βy T.P. = −0·761 S.E. = ±0·673 t = −1·131 N.S.
Wool production × Photoperiod, keeping air temperature constant	βy T.P. = +0·055 S.E. = ±0·673 t = +0·082 N.S.

R = 0·711*

Multiple regression equation:

$Y = 24{\cdot}591 - 0{\cdot}319\ x_1 + 0{\cdot}117\ x_2$

where, Y = Wool production in mg. per cm.² of body area for 30·4 days,

x_1 = Air temperature in degrees Centigrade,

and x_2 = Photoperiod (duration of day length) in hours.

N.S. = Not significant. *$P < 0{\cdot}05$

Bikaneri sheep. The air temperature was negatively correlated with wool production, 50·8 per cent of the variation in wool production being due to it. Similarly, the wool production was negatively and linearly correlated with day length, 44·49 per cent of the variation in wool production being due to the variation in day length. Wool production increased with decreasing photoperiod, i.e., the duration of day length in hours. The partial correlation coefficients between wool production and the two climatic factors, air temperature and photoperiod, obtained on keeping the other factor constant were found to be not significant. The values of partial regression coefficients were also found to be not significant, whereas the multiple correlation coefficient between air temperature, photoperiod and wool production was found to be statistically significant ($P < 0{\cdot}05$). This shows that the wool production in Bikaneri sheep is influenced jointly by environmental temperature and photoperiod and not independently, and that they control the variation in the wool production to an extent of 50·6 per cent. The rest may be due to genetical and other possible environmental factors. The above conclusion is also in conformity with the finding that both temperature and photoperiod were significantly and positively correlated with each other ($P < 0{\cdot}001$). The correlation coefficient between maximum daily air temperature and wool production was also found to be significant, though of smaller magnitude in comparison to mean air temperature. Hence, it is seen that the variation in the mean daily air temperature had more profound effect on the wool production than the variation in the maximum daily air temperature.

The results obtained in the present investigations are quite in harmony with the physiological principles of metabolism. Thus, many authors have reported that wool growth is influenced by thyroid secretions (Miller, 1931; Marston and Pierce, 1932; Hart, 1954; Maqsood, 1950, 1955; Ferguson, 1951, 1958; Labban, 1957a, b; Carter, 1958; Godfrey and Tribe, 1959; Kirton *et al.*, 1960; Underwood, 1960). It is known that high environmental temperatures reduce thyroid secretions (Bogart and Mayer, 1946; Lee and Phillips, 1948; Lee, 1949; Blincoe and Brody, 1955a, b; Graham *et al.*, 1959). The results obtained in the present experiment are at variance from those reported by some workers in other countries. Ferguson *et al.* (1949) found that the wool

production was positively correlated with environmental temperature. Wodzicka (1960) obtained higher wool yield in Columbia-Southdale, Merino and Hampshire rams when environmental temperature was high. Daly and Carter (1955, 1956) observed significant positive correlation in wool production and temperature in the Lincoln sheep, positive trend in Corriedale and Fine Merino, and consistently negative in the Polwarth breed. Coop (1953) working with Corriedale ewes disapproved the theory of vasodilation put forth by Ferguson *et al.* (1949), as he did not find any difference due to artifically raised temperature. He even suggested that the low wool production in winter might be due to the effect of high temperature in the previous summer, which might have depressed the thyroid activity and the late revealation of reduction in wool growth might have been due to time lag. Lee (1949, 1950) cited 21°C as the lower limit of thermoneutrality in sheep though they could thrive at temperatures as low as −40°C. In most of the temperate regions from where the above-mentioned reports regarding decreased wool production with fall in temperature have emanated, the temperature in winter is usually lower than 21°C and the summer temperature is not as extreme as in the tropical regions. From this view-point it is quite appropriate that the workers in the temperate regions observed positive correlation between wool production and environmental temperature.

There have been no reports so far correlating the diurnal photoperiod with the wool production in sheep, excepting the results presented in this paper and those obtained from the findings under laboratory conditions that with decreased photoperiod there was increased wool production (Hart, 1953, 1961; Coop and Hart, 1953; Wildman, 1957, 1958).

The results obtained in the present experiment indicate that vapour pressure, i.e., atmospheric humidity, has no effect on wool production. Bowstead and Larose (1938) and Daly and Carter (1955) obtained similar results. Lee (1950) stated that humidity had a marked effect only at high temperatures, which is concurrent with our results. It can be seen that in periods 6 and 7, when there was high vapour pressure accompanied by high environmental temperature, the wool production was depressed (Fig. 3).

SUMMARY

The wool production in Bikaneri sheep is markedly affected by climatic factors. The air temperature and the photoperiod have been found to jointly influence wool production to an extent of nearly 51 per cent. Humidity does not exert any significant influence on wool production. But it is considered that high environmental humidity accompanied by high temperature exerts physiological stress on animals (as in autumn), consequently reducing their productivity.

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EFFECT OF ENVIRONMENTAL FACTORS ON WOOL GROWTH

II. EFFECT OF PHOTOPERIODICITY

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In recent times much work has been done on the effect of the photoperiodicity on animal production by controlling the duration of the light or darkness under laboratory conditions. Marshall (1937) was one of the earliest workers who drew the attention to the effect of photoperiodic environment in the transfer of sheep from one locality to another. Yeates (1954) stated that the knowledge of the effect of photoperiodicity on animals has opened a new avenue in the field of animal breeding. In the improvement of animals it is necessary to select types and breeds having gene combinations likely to fit a certain environment. India, being a vast country, the variation in day length is of an appreciable amount and it has a bearing on sheep improvement programmes from the ecological point of view.

MATERIAL AND METHODS

This work was based on the same flock of Bikaneri ewes described earlier (Nagarcenkar and Bhattacharya, 1964). The three groups of ewes were randomly allotted, after the standardization period, to receive different photoperiodic treatments. L_1 group was given light : darkness ratio of 8 hours light : 16 hours darkness, L_2 group of 16 hours light : 8 hours darkness, and the third group served as control and received the normal diurnal day light : darkness ratio (Nagarcenkar and Bhattacharya, 1964). The photoperiodic treatment (comparative period) lasted for 180 days from June 19, 1959.

The Control group was housed in a paddock screened from direct sunlight. The other two experimental groups were kept independently in two cement concrete-floored rooms, each of dimensions 24·5 ft. × 24·5 ft. Both the rooms were light-proof. The L_1 group receiving light : darkness ratio of 1:2 was kept in a psychrometric chamber with ceiling height 8 ft. 10 inch. The light period in this room was from 6 A.M. to 2 P.M. continuously. The L_2 group receiving light : darkness ratio of 2:1 was kept in an adjacent room with ceiling height 9 ft. 10 inch, which was converted into a darkroom. The light period in the latter room was from 6 A.M. to 10 P.M. continuously. The light in each of these two rooms was from four 3·5 ft. 40-watt Phillips fluorescent day-light tubes suspended one foot from the ceiling equidistant from each other. The radiation as measured by G. E. radiation metre was 0·025 G. Cal./sq. cm./min., i.e., 15 K cal./sq. m./hr. at sheep's eye level throughout the room.

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The radiation in the control pen was also on an average nearly the same due to the screening of the walls. The psychrometric room had an exhaust fan fitted to remove the expired air. The inlet ducts were kept open so that there was replacement of fresh air and the average air temperature was maintained similar to the atmospheric air temperature. In the adjacent room an exhaust fan was fitted in the wall to remove the expired gases and an electric table fan was kept in a corner to replenish fresh air. The air temperature in this room was also almost similar, though slightly higher than the outside temperature; the difference in temperature over the total Comparison period of 180 days was 2·03°C. There was an observation window between the psychrometric chamber and the adjacent room and the same was left open when there was light or dark period in both the rooms and kept closed from 2 P.M. to 10 P.M. The evening cleaning, feeding and watering in the psychrometric chamber which housed the L_1 group ewes was finished in time to start the darkness period as per schedule.

Immediately after the completion of the Comparison period on 16th/17th/18th December, 1959, the L_1 and L_2 groups were removed from their rooms and housed in thatched pens similar to that of the Control group. The post-comparison period lasted for 90 days. Two ewes (Nos. 45 and 94) from L_1 group got accidentally mated to a ram. These happened to be trio mates of ewes (Nos. 84 and 249) from Control group eliminated earlier. Therefore, while analysing the results on wool production, the ewes from trios 11 and 12 were omitted; the results are thus based on trios 1 to 10, i.e., ten ewes in each group and 30 ewes in all.

RESULTS

The data for each period on the three groups of ewes, viz. Control, L_1 and L_2, have been presented in Table I. The same are summarized for different periods, namely, Standardization period, Comparison period, and post-Comparison period in Table II.

TABLE I. AVERAGE WOOL PRODUCTION OF THE THREE GROUPS OF EWES DURING THE EXPERIMENT

(Wool production mg./cm.^{2}/30·4 days)

Groups	Periods												
	P_1	P_2	P_3	P_4	C.P.$_1$	C.P.$_2$	C.P.$_3$	C.P.$_4$	C.P.$_5$	C.P.$_6$	P.C.P.$_1$	P.C.P.$_2$	P.C.P.$_3$
Control	13·41	18·81	13·31	17·08	17·50	16·26	16·10	17·21	19·69	22·47	21·94	19·26	19·42
L_1	13·69	18·13	15·36	17·74	18·12	20·43	20·24	20·26	18·68	25·24	25·52	24·05	23·94
L_2	13·54	18·50	14·79	18·09	19·44	17·98	17·64	19·72	20·35	23·82	26·18	23·33	19·45

Statistical analysis of the data for the Comparison period was carried out and the summary of the analysis of variance is presented in Table III. The variance due to Treatments, Blocks (Trios of ewes), Periods, and interactions between Treatments × Blocks and Blocks × Periods were all statistically highly significant. Testing further

TABLE II. AVERAGE WOOL PRODUCTION OF THE THREE GROUPS OF EWES DUE TO THE EFFECT OF TREATMENTS

(Wool production in mg./cm.^{2}/30·4 days)

Groups \ Periods	standardization period (60 days)	Comparison period (180 days)	Post-Comparison period (90 days)
Control	16·11	18·20	20·21
L_1	15·91	20·49	24·50
L_2	16·02	19·82	22·99

the Treatment variance against the interaction variance of Treatments × Periods, the F value 6·18 was found to be statistically significant at 5 per cent level. Thus, showing that the treatments were effective in all periods, i.e., irrespective of the seasons, as varying from mid-June (summer) to mid-December (winter). The variance due to Periods was also tested against the variances due to interaction between Treatments × Periods and Blocks × Periods, and the F values found were 10·85 and 3·12, respectively, which were both highly significant statistically ($P<0·01$). Thus, there was significant seasonal variation in wool production in spite of the photoperiodic treatments.

TABLE III. SUMMARY OF ANALYSIS OF VARIANCE FOR WOOL PRODUCTION DUE TO DIFFERENT PHOTOPERIODIC TREATMENTS

Source of variation	D.F.	S.S.	M.S.	F ratio
Total	179	5,631·98		
Treatments	2	166·07	83·03	10·73**
Blocks (Trios)	9	1,692·08	188·01	24·30**
Periods	5	728·48	145·70	18·83**
Interactions				
Treatments × Blocks	18	2,004·77	111·38	14·40**
Treatments × Periods	10	134·33	13·43	1·74 N.S.
Blocks × Periods	45	210·01	46·67	6·03**
Error	90	696·25	7·74	

N.S. = Not significant. **$P<0·001$

The Critical Difference (C.D.) values were calculated to see if the differences in the wool production of ewes due to the photoperiodic treatments were significant. The data are given in ascending order in Table IV.

TABLE IV. AVERAGE WOOL PRODUCTION IN EWES DUE TO THE DIFFERENT PHOTOPERIODIC TREATMENTS

Group	Average wool production (mg./cm.²/30·4 days)	C.D. values
Control	18·20	At 5% level 1·11
L_2	19·82	At 1% level 1·34
L_1	20·49	At 0·1% level 1·73

Differences between the values under the same bar are not satistically significant.

It will be seen from Fig. 1 that the Blocks (Trios of ewes) yielded varying results in the Comparison period due to the effect of treatments, though they were grouped on

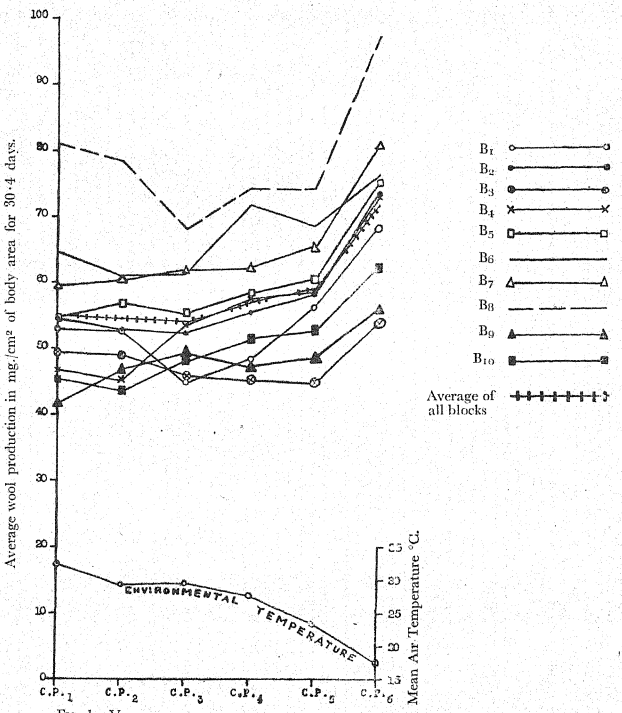

FIG. 1. VARIATION IN THE WOOL PRODUCTION DURING THE COMPARISON PERIOD

the basis of their average wool production during the Standardization period. The Homogeneity test was, therefore, conducted on the basis of the wool yield of each Block (Trio) during the Standardization period. This has been presented in Table V.

TABLE V. TEST OF HOMOGENEITY OF THE BLOCKS
(Trios of ewes)

Blocks	B_1	B_2	B_3	B_4	B_5	B_6	B_7	B_8	B_9	B_{10}
Average wool production (mg.)	15·23	15·89	16·35	17·68	19·63	18·55	14·83	16·35	13·67	11·98

Average of all blocks 16·02. Standard deviation 2·27

It can be seen that all the trios are homogeneous and their average wool production values were between the average ± 2 S.D. values, i.e., between 16·02 ± 4·54 mg.

DISCUSSION

The wool production in both the treatment groups, L_1 and L_2, increased over the Control group of ewes. The analysis of variance of the data revealed that the photoperiodic treatments had significant effects which were manifested irrespective of the seasonal variation in climatic conditions. The reports available on the effect of photoperiodicity on wool production indicate that wool production increased as the duration of photoperiod reduced (Hart, 1953, 1961; Coop and Hart, 1953; Wildman, 1957, 1958). Wodzicka (1960), who worked with Columbia × Southdale, Hampshire and Merino rams at Beltsville (U.S.A.), however, stated that there was no effect of photoperiodicity on wool production. She questioned the results of Hart (1953) and Coop and Hart (1953), as these were based on a few animals and because the data were not subjected to statistical analysis. Wodzicka's experimental plan may also be criticised, as she did not assess the inherent normal wool production in the experimental animals before allotting them to the different groups to receive the various treatments. As it is well-known that there can be large variations between individual animals within apparently homologous flock, it is absolutely necessary while studying a complex physiological phenomenon such as wool production to eliminate all possible variations. Although Wodzicka had five rams in each group, as these belonged to three different breeds, another complicating factor-breed × treatment interaction was introduced. Thus, her results were actually based on two animals each of Columbia × Southdale and Merino breeds, and one animal of Hampshire breed. The data presented by Wodzicka revealed varying wool production in the individual animals of Columbia × Southdale and Merino breeds. It is not possible to judge whether the differences observed were due to inherent wool production rates or gene × treatment interactions. Lastly, the animals though managed similarly, were not fed on a constant ration throughout the year. The results and conclusion drawn thereon by Wodzicka are, therefore, liable to criticism.

Yeates (1954) suggested that the tropical light environment did not produce any effect on animals' coat cycles. However, the results obtained by the authors as well as by Symington (1959) have conclusively proved that it does produce some effect. Symington (1959) worked at Salisbury (Lat. 17°50'S) on Persian Blackhead ewes. Subjecting one group to controlled light treatment of 14 hr darkness : 4 hr light followed by 2 hr darkness : 4 hr light he found that treated ewes grew a longer and dense coat. In another experiment, when a group was given reduced light intensity of the same duration as the control, it was observed that the coat also became long and dense. It is not fully known as yet how day light differs from the artificial light in its effects on animal's metabolic activity and hormonal output (resulting finally in production). Since Hart (1953) and Coop and Hart (1953) gave part of the light exposure in sunshine and part in the pens by artificial means, introducing an additional complex their factor, results should be accepted with caution.

How actually the effect of photoperiodicity is manifested is not yet quite clear, although it is thought that light stimulates the pituitary through the eye acting as receptor and threby transmitting the impulses neurally to the hypothalamus. Most of the reports have suggested that the shorter day length induced greater pituitary stimulation and thereby larger wool production was obtained. It was also believed that the light of various intensities did not yield different effects. The results obtained by Symington (1959) are quite contrary. In the present study, wool growth in L_2 group ewes having 16 hr. light continuously increased over the Control group ewes receiving diurnally varying day length with an average of 13·198 light hours during the same period. Ryder (1956) observed in Masham sheep that wool production increased as the day length increased. Hammond Jr. (1951) suggested that both light as well as darkness may stimulate oestrus in ferrets. Yeates (1955) observed that on reversing the photoperiod hair growth increased significantly in growing Poll Shorthorn calves. This shows that a mere change of photoperiod from the normal stimulated increased production. Stegenga (1960) working with three pairs of identical twins of the Dutch red and white cattle breed found that on increasing the photoperiod the hair growth reduced for some time and then it increased. However, his results were not quite conclusive, as the animals were immediately put on a reduced plane of light. Henderson *et al.* (1958) also obtained conflicting results working with ewes maintained on different photoperiods. They observed significantly greater thyroid activity in ewes on 12 hr. light period as compared to those on 8 and 16 hr. light periods.

When the results in Table I are considered in conjunction with the day length duration (Nagarcenkar and Bhattacharya, 1964; Fig.2) it is seen that the animals were allotted to different photoperiodic treatments when they were on naturally increasing plane of day light duration, the longest day of the year (June 21) following two days after the animals were on the different photoperiodic treatments. Thus, for group L_1, there was a sudden decrease in photoperiod, whereas for group L_2 there was a further increase. The effect of this is obvious from the data on wool production in periods $C.P._1$, $C.P._2$ and $C.P._3$ (Table I). It reveals a decreasing trend in L_2 group, whereas the L_1 group shows a spurt in production. Similarly, on discontinuance of the treatments, the animals in L_2 groups faced a reduced photoperiod, which was declining till December 22 and then rose gradually, though the day length duration

was shorter than during the Comparison period. The L_1 group on the contrary faced an increased photoperiod. The examination of the data on wool production in the post-comparison period shows that due to the reduced photoperiod in L_2 group the wool production was immediately stimulated and later on started reducing with increasing photoperiod, whereas in the L_1 group it was not so contrastingly affected and was going down gradually. This response of the L_1 group may be said to be due to the delayed expression of the stimulus already received during Comparison Period. Wildman (1957) has made similar observations in his work with Romney rams.

From the response of the Bikaneri sheep to the different photoperiodic treatments and especially the increased wool production of L_2 group over that of the Control group, it may be postulated that any change in the normal diurnally varying photoperiod stimulates the pituitary and an increased wool production results. The work of Stegenga (1960), though inconclusive, supports this contention. As already stated above, no data are available regarding the physiological differences that might be produced by the artificial photoperiods as compared to the natural day light; hence, it may be suggested that any artificial photoperiod may stimulate the pituitary. There is no report of investigation on the effect of a large photoperiod as used in the present work on wool production in sheep, except that of Hart (1961), wherein he gave additional artificial light after sunset to four Corriedale ewes in New Zealand for a period of 12 months and did not find any difference in wool growth as compared to control. The reason for his results could be that while during daytime the ewes received 50 f.c. light intensity, at night (with artificial light) they had only 5 f.c. light intensity. This tremendous difference in intensity might have acted as contrast sensitization for sheep's receptor mechanism, same as in case of *light-darkness*. Hart (1961) was also perplexed due to the results obtained under natural environment, wherein he found maximum rate of wool growth during period of increasing day light. This may be conjectured to be due to the greater effect of atmospheric temperature as compared to that of photoperiod on wool production (Nagarcenkar and Bhattacharya, 1964). Nagarcenkar (1963) also reported that the atmospheric temperature in the summer in temperate countries is in the zone of thermoneutrality of sheep, thus, maximum wool growth in sheep being manifested during that season. The period of maximum wool growth in New Zealand as observed by Hart (1961) was December-February, which is summer season in the Southern hemisphere.

The Control group of sheep in the present investigations were housed in a thatched shed with split bamboo wall and were exposed to all the rigours of weather conditions, such as cold biting winds in the winter and scorching winds in the hot summer. The sheep in other two groups were comparatively comfortable because of the protection afforded by brick walls. An intense shedding of fibres was also observed in the L_2 group ewes. Hence, it may be stated that the wool yield would be evidently lesser in animals subjected to a large photoperiod for a prolonged duration. There was significant Periods effect and also Treatments $\times$ Blocks and Blocks $\times$ Periods interactions (Tables II and III). Wildman (1957) also observed Groups $\times$ Periods interaction. The Periods effect was necessarily due to the effect of other varying climatic factors, especially the effect of temperature. Because of the significant variation observed in the different Blocks, homogeneity test was carried, which

revealed that the Blocks were all homogeneous. Hence, the differences may be said to be due to the genetic variability in the animals. The Blocks × Periods and Blocks × Treatments interactions can be alleged to be due to the same reason. Hammond (1950), Dunlop (1951), Hafez (1952), Hancock (1953), and King and Young (1955) either suggested or proved conclusively that the individual reactions of animals to different environmental treatments vary, purporting thereby gene × environment interaction.

SUMMARY

The effect of different photoperiods on wool production was studied in 30 Bikaneri ewes. It was observed that in both the groups of ewes provided with 1:2/light: darkness rhythm and 2:1/light:darkness rhythm, the wool production increased significantly than in the Control group of ewes.

It is postulated that the artificial photoperiod, as compared to the natural day light, generates quite different responses in the animals and that any change of photoperiod from the normal stimulates the pituitary, which finally results in greater wool production. Intense shedding of fibres was, however, observed in the group subjected to larger photoperiod and, hence, it is concluded that due to this the actual wool yield may get reduced in the animals receiving larger photoperiod for a prolonged duration.

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SEXUAL BEHAVIOUR OF BULLS IN RELATION TO OESTRUS DISTRIBUTION IN FEMALES

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REGULARITY in the sexual performances of bulls irrespective of the differences in climate, season and geographic region, would be a help to facilitate the efficient working of artificial insemination centres in the country. Many workers (Erb *et al.*, 1942; Mukerjee and Bhattacharya, 1952; Kushwaha *et al.*, 1955; Singh and Husain, 1958) in the past have tried to correlate the sexual performances including semen quality of bulls with different seasons of the year. Tomar and Mittal (1960), Kohli and Suri (1960) and Malik *et al.* (1960) reported that Hariana and Sahiwal cows are not seasonal breeders like buffalo-cows, which have been observed to follow seasonality (Arunachalam *et al.*, 1952; Tomar and Tomar, 1960; Kohli and Malik, 1960).

In the present study attempts have been made to observe the seasonal and regional effects on the semen volume and to correlate them with the oestrus frequency in females of the respective breeds.

MATERIAL AND METHODS

The data for this work were taken from the artificial insemination centres of different regions in Uttar Pradesh, viz., eastern region (extreme moist heat region), western region (dry heat and lower rainfall region) and Bundelkhand region (extreme dry heat region). The data of Sahiwal breed were available only from the eastern region. Only the first available performance of the bulls for one year was considered for bringing uniformity in the data. The particulars are given below.

Breed	No. of bulls	No. of semen ejaculation records	No. of cows in heat
Hariana	60	3,298	2,669
Sahiwal	17	943	915
Murrah	70	4,404	1,476

The care, feeding and management of the bulls were practised according to the standards as laid down by the Animal Husbandry Department, Uttar Pradesh. In all the centres semen was invariably collected twice a week.

The data were arranged month-wise, season-wise and region-wise, and were analysed according to the methods by Snedecor (1956).

RESULTS AND DISCUSSION

The data on the average number of semen ejaculations, average volume of semen per ejaculate and total volume were tabulated month-wise for all the three breeds (Table I). The average volume of semen donated by Hariana and Sahiwal bulls

TABLE I. MONTH-WISE DISTRIBUTION OF THE FREQUENCY AND THE VOLUME OF SEMEN EJACULATES OF BULLS

Months	Total No. of ejaculates	Average No. of ejaculations per bull	Average semen volume per ejaculation (ml.)	Average vol. of semen per bull (ml.)	Percentage of total vol. of semen	Total semen volume (ml.)
			Hariana bulls			
January	253	4·22	4·08±0·51	17·07	7·44	1,024·50
February	246	4·10	4·14±0·61	16·84	7·33	1,010·40
March	287	4·72	4·59±0·67	20·69	8·79	1,211·25
April	299	4·98	4·38±0·61	21·12	9·19	1,266·90
May	319	5·32	4·41±0·63	22·28	9·70	1,336·65
June	278	4·63	4·61±0·65	20·06	8·73	1,203·50
July	288	4·80	4·73±0·68	20·50	8·92	1,229·40
August	273	4·55	4·42±0·65	19·04	8·22	1,132·25
September	270	4·50	3·70±0·54	16·92	7·37	1,015·05
October	254	4·23	4·52±0·65	19·38	8·44	1,162·20
November	283	4·72	4·69±0·69	19·40	8·45	1,163·80
December	248	4·13	4·31±0·68	17·04	7·42	1,022·20
			Sahiwal bulls			
January	67	3·94	4·61±1·45	18·16	7·48	308·8
February	65	3·82	4·45±1·37	17·29	7·12	293·9
March	70	4·12	4·28±1·27	17·64	7·26	299·9
April	89	5·23	4·57±1·22	23·95	9·86	407·1
May	105	6·18	4·37±1·19	27·02	11·13	459·4
June	83	4·88	4·54±1·24	22·17	9·13	376·9
July	85	5·00	4·50±1·27	22·51	9·27	382·6
August	85	5·00	4·56±1·39	22·81	9·39	387·8
September	80	4·06	3·98±1·16	18·76	7·72	318·9
October	67	3·94	4·30±1·35	16·95	6·98	288·1
November	71	4·18	4·13±1·34	17·26	7·10	293·5
December	76	4·47	4·11±1·34	18·36	7·56	312·2

TABLE I—(*Concld.*)

Months	Total No. of ejaculates	Average No. of ejaculations per bull	Average semen volume per ejaculation (ml.)	Average vol. of semen per bull (ml.)	Percentage of total vol. of semen	Total semen volume (ml.)
			Murrah bulls			
January	492	7·02	2·79±0·06	19·56	11·03	1,388·70
February	416	5·94	2·84±0·44	16·40	9·52	1,180·80
March	336	4·80	3·02±0·52	14·08	8·17	1,013·50
April	237	3·39	3·15±0·47	10·38	6·02	747·10
May	248	3·54	3·12±0·49	10·78	6·25	775·90
June	208	2·97	2·97±0·48	8·60	4·99	618·80
July	241	3·44	2·81±0·45	9·44	5·48	679·45
August	305	4·36	2·86±0·39	12·11	7·03	872·00
September	437	6·24	2·64±0·40	15·86	9·21	1,141·75
October	511	7·30	2·55±0·40	18·13	10·52	1,305·50
November	508	7·26	2·69±0·41	18·98	11·02	1,366·45
December	485	6·93	2·75±0·47	18·53	10·76	1,334·70

per ejaculate was found to be 4·38±0·18 and 4·37±0·37 ml. respectively. Shukla and Bhattacharya (1949) estimated the averages as 3·16±0·2 and 3·8±0·35 ml. for the respective breeds. The average number of collections per month for both the breeds is 4·6. The same data when analysed for differences in months, seasons as well as regions (Tables II and III) indicate that the differences in the frequency of semen ejaculations and its total volume were statistically not significant.

Erb *et al.* (1942), working with the Holstein, Jersey, Guernsey and Ayrshire bulls, observed that the average semen volume produced was least in July to September period. In the present study on the Hariana bulls there was lowered output of semen from August to February, and in case of Sahiwal bulls from October to March.

The Murrah buffalo-bulls showed a highly significant effect of month and season of the year on the semen donation frequency and its production quantum (Tables II and III). The highest number of ejaculations was obtained within the months of October to February. The months of October and June recorded the highest and the lowest collection frequencies (7·3 and 2·9) respectively. However, there was no significant difference in the per ejaculate semen volume in different months. Kushwaha *et al.* (1955) also obtained similar results with the buffalo-bulls. In the present analysis the higher volume obtained in the months from October to February was due to the increase in the ejaculation frequencies, which a buffalo-bull normally could do

TABLE II. ANALYSIS OF VARIANCE OF THE EFFECTS OF SEASONS AND MONTHS ON THE FREQUENCY AND VOLUME OF SEMEN EJACULATIONS

Source of variation	Hariana			Sahiwal			Murrah		
	d.f.	M.S. (frequency)	M.S. (volume)	d.f.	M.S. (frequency)	M.S. (volume)	d.f.	M.S. (frequency)	M.S. (volume)
				Seasons					
Among seasons	3	N.S. 33·96	N.S. 1,008·88	3	N.S. 39·02	N.S. 1,129·56	3	1,577·90**	13,209·31**
Within season	236	58·10	1,268·47	64	109·99	2,261·21	276	90·70	942·82
Total	239			67			279		
				Months					
Among months	11	N.S. 9·02	N.S. 250·62	11	N.S. 8·03	N.S. 143·45	11	195·02**	1,151·33**
Within month	708	8·13	178·14	192	13·76	288·00	828	13·57	127·02
Total	719			203			839		

N.S.=Not significant. **=Significant at P<00·1

TABLE III. ANALYSIS OF VARIANCE SHOWING ZONAL EFFECT ON THE FREQUENCY AND VOLUME OF SEMEN EJACULATES OF HARIANA AND MURRAH BULLS

Source of variation	Hariana			Murrah		
	d.f.	M.S. (frequency)	M.S. (volume)	d.f.	M.S. (frequency)	M.S. (volume)
		Eastern region				
Among months	11	N.S. 4·48	N.S. 39·47	11	106·07**	1,202·43**
Within month	120	8·21	199·29	276	12·92	94·71
Total	131			287		
		Bundelkhand region				
Among months	11	N.S. 6·76	N.S. 9·59	11	31·02**	171·11**
Within month	108	5·29	178·74	48	4·50	22·22
Total	119			59		
		Western region				
Among months	11	N.S. 8·33	N.S. 240·38	11	83·38*	417·63*
Within month	456	8·68	179·22	480	14·62	166·29
Total	467			491		

N.S.=Not significant. **=Significant at P<0·01

because of the favourable season. The average number of semen collection frequency in the Murrah-bulls was 5·2 per month per bull. The average volume of semen per ejaculate was recorded to be $2{\cdot}81 \pm 0{\cdot}13$ ml. Shukla and Bhattacharya (1949), however, estimated it to be $1{\cdot}8 \pm 0{\cdot}1$ ml. in the buffalo-bulls.

The fact that the average volume of semen per ejaculation in all the three breeds was not found variable according to the months and the seasons of the year indicate that the capacity of an individual bull to donate semen in each ejaculation is more or less constant. What season or the month seems to effect is the effective desire for mating. Sex libido is indicated to be constant in the Hariana and Sahiwal bulls, whereas it is predominantly exhibited by the Murrah bulls in the months from September to March.

Oestrus in females: The Hariana cows, which calve more or less uniformly throughout the year (Tomar and Mittal, 1960; Kohli and Suri, 1960), when analysed for the oestrus distribution were found to be uninfluenced by the months or seasons of the year (Table IV). Sahiwal cows, which did not show any month-to-month significant variation, when grouped into four seasons indicated a seasonal distribution of oestrus, significant only at $P<0{\cdot}05$ (Table IV). During autumn and winter they exhibited significantly lower distribution of oestrus (18·25 and 17·60 per cent respectively).

TABLE IV. FREQUENCY OF OESTRUS IN HARIANA, SAHIWAL AND MURRAH BUFFALO-COWS

Oestrus distribution	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
						Hariana cows							
No. of cows in heat	226	252	285	264	271	250	210	195	174	195	170	177	2669
Percentage distribution	8·47	9·44	10·68	9·89	10·15	9·37	7·87	7·31	6·52	7·31	6·36	6·63	100·00
χ^2 value	0·002	0·143	0·663	0·292	0·398	0·130	0·030	0·125	0·393	0·125	0·461	0·347	N.S. 3·114
Seasonal distribution	Spring (Feb. to Mar.)			Summer (May to July)			Autumn (Aug. to Oct.)			Winter (Nov. to Jan.)			
Percentage	30·01			27·39			21·13			21·47			100·00
χ^2 value	0·8364			0·2085			0·7088			0·5804			N.S. 2·3341
						Sahiwal cows							
No. of cows in heat	56	75	92	111	116	115	78	53	57	57	54	51	915
Percentage distribution	6·12	8·21	10·06	12·12	12·68	12·57	8·52	5·79	6·23	6·23	5·90	5·57	100·00
χ^2 value	0·586	0·002	0·359	1·724	2·272	2·158	0·043	0·775	0·529	0·529	0·709	0·914	N.S. 10·600
Seasonal distribution	Spring (Feb. to Apr.)			Summer (May to July)			Autumn (Aug. to Oct.)			Winter (Nov. to Jan.)			
Percentage	30·38			33·77			18·25			17·60			100·00
χ^2 value	0·9527			2·2776			2·4966			3·1114			8·8383*

TABLE IV—(*Concld.*)

Oestrus distribution	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
						Murrah buffalo-cows							
No. of cows in heat	222	175	77	59	44	30	41	61	127	200	209	231	1476
Percentage distribution	15·04	11·86	5·22	3·99	2·99	2·03	2·78	4·13	8·60	13·55	14·16	15·65	100·00
χ^2 value	5·405	1·496	1·611	2·261	3·423	4·765	3·698	2·117	0·009	4·765	5·108	6·482	40·09**

Seasonal distribution	Spring (Feb. to Apr.)	Summer (May to July)	Autumn (Aug. to Oct.)	Winter (Nov. to Jan.)	
Percentage	21·07	7·79	27·37	43·77	100·00
χ^2 value	0·7330	38·0211	0·1614	8·0492	46·965**

N.S.=Not significant. *=Significant at $P<0·05$ **=Significant at $P<0·01$

Tomar and Tomar (1930) observed that Murrah buffalo-cows were highly seasonal in their calving activities and that 77·77 per cent cases of the calvings occurred from July to November. The present data also revealed a highly significant seasonal and month-to-month distribution of oestrus in buffaloes (Table IV). The highest percentage of oestrus was observed during winter (43·77). In summer (May to July), however, only 7·79 per cent buffaloes were found in heat. The most active period when the highest percentage of the buffaloes (79·27 per cent) were observed in heat was that from September to February.

Oestrus activities of the cows vs. *seasonal response of the breeding bulls:* Hariana cows appear to come in heat irrespective of the month and/or season of the year. However, higher distribution of the oestrous cows occurred during the period from January to June (58 per cent). Hariana bulls on the contrary showed an insignificant month-to-month variation in their semen ejaculation behaviour, which could be a right index of the sex libido. It was also revealed from the present analysis that 51·18 per cent of the total volume of semen donated by the bulls was available during the more active period of oestrus in cows (January to June).

In the case of Sahiwal breed the cows were more active during February to July (64·15 per cent) and the bulls were also alike in donating comparatively higher percentage of semen (53·77 per cent) during the same period of the year. The significant difference indicated by oestrus frequency at $P<0·05$ level may be because of the sampling error in the investigation.

The sexual behaviour of Murrah buffalo-cows and that of the bulls was synchronizing each other significantly more than that observed in the case of Hariana and Sahiwal breeds. The higher distribution of the oestrus in the female (78·86 per cent) was observed during the period falling between September to February; similarly, the percentage of semen donated by the bulls (62·06 per cent) was comparatively more during the afore-said period.

The results indicate that the reproductive function of both males and females is uniform in response to seasonal variation (Fig. 1), i.e., the breeds of cattle are not effected by seasonal changes, whereas in buffaloes, both male and female are equally effected by such seasonal variation. Thus, any corrective method either in management or feeding practices would equally be effective in both the sexes of buffaloes.

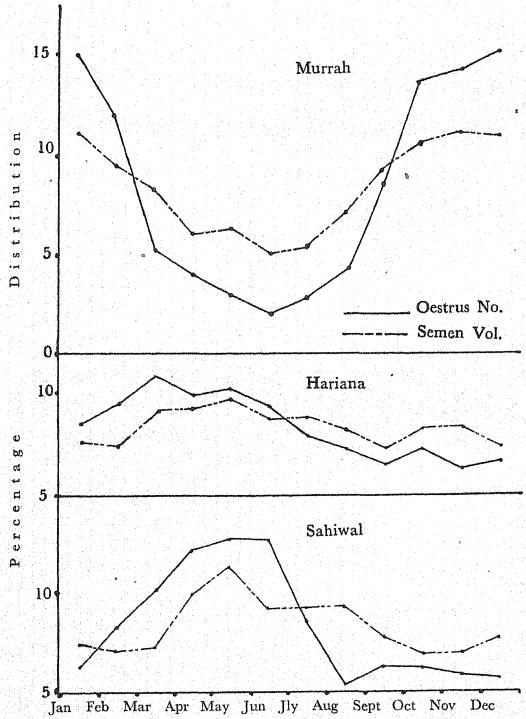

FIG. 1. EFFECT OF THE MONTH ON THE FREQUENCY OF SEMEN VOLUME IN RELATION TO OESTRUS IN FEMALES

SUMMARY

Analyses of 3,298, 943 and 4,404 semen ejaculations of 60 Hariana, 17 Sahiwal and 70 Murrah bulls, respectively, were done to find out the month-to-month and

seasonal variations in the ejaculation frequencies and semen volumes. Bulls of Hariana and Sahiwal breeds did not show any significant effect of month and season of the year on the semen ejaculation frequencies and the total semen output. No such variations were observed in the Hariana and Sahiwal cows with regard to oestrus frequencies. On the contrary, the Murrah bulls and cows were found to be least reactive during the hot season when the air was less humid.

The average frequencies of ejaculations in Hariana, Sahiwal and Murrah bulls per month were 4·6, 4·6 and 5·2, respectively, and the average volume of semen per ejaculation was found to be $4{\cdot}38 \pm 0{\cdot}18$, $4{\cdot}37 \pm 0{\cdot}37$ and $2{\cdot}81 \pm 0{\cdot}13$ ml. in the respective breeds.

The sexual performances of both males and females in all the breeds studied were alike in different months and seasons of the year.

ACKNOWLEDGEMENTS

The authors are thankful to Sri C.V.G. Choudary, Principal of the U.P. College of Veterinary Science and Animal Husbandry, Mathura, for kindly providing the facilities, and to Sri Dharmendra Kumar, Statistician, for his help and co-operation.

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OBSERVATIONS ON THE HAEMOLYTIC ACTIVITY OF NEWCASTLE (RANIKHET) DISEASE VIRUS

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THE haemolytic property, which is often exhibited by many bacteria, was for the first time shown to be possessed by mumps virus by Morgan *et al.* (1948). An haemolysin associated with NDV was described in the subsequent year by Kilham (1949). Burnet and Lind (1950) and Liu (1952) observed that calcium-ions inhibited the haemolytic activity of NDV. Burnet and Lind found that the haemolytic activity was greatly increased by methonol precipitation of virus preparations, while Liu obtained the same effect by freezing and thawing. Chu and Morgan (1950a) and Morgan (1951) made similar observations in the case of mumps virus. Nilakantan *et al.* (1963) studied the haemolytic activity of NDV strains isolated in India and the observations made on the haemolytic activity of a few strains of NDV of varying virulence are presented here.

MATERIALS AND METHODS

Virus strains: Six strains of NDV, namely, the Mukteswar (R_2B), 'F', B_1, Palestine, KB_{12} and 'V' described by Nilakantan *et al.* (1961), were used.

Virus pools: The pooled allanto-amniotic (a.a.) fluid collected from embryonating eggs infected with the different strains of the virus was centrifuged at 2,500 r.p.m. for ten minutes and stored in the freezing chamber of a refrigerator. This formed the source of virus for the study.

Freezing and thawing of a.a. fluid: Allanto-amniotic fluid preparation of the virus in pyrex tubes was allowed to freeze at –4°C in the freezing chamber of a refrigerator. Soon after freezing had occurred, the tubes were transferred to a water bath at 56°C and the contents allowed to thaw for three to five minutes. The freezing and thawing processes were repeated three times.

Dialysis of a.a. fluid: The virus preparation after freezing and thawing was dialysed in a cellophane bag. The bag was placed in a large volume of 0·001M phosphate buffer saline at pH 6·6. Dialysis was allowed to proceed at 4°C for about 36 hours with the buffer saline being changed every 12 hours.

Test: Preparation of red blood cells and the method of carrying out haemolysis test with virus preparations was similar to that adopted by Nilakantan *et al.* (1963). The degree of haemolysis was estimated with lumetron photoelectric colorimeter using green filter. The results were expressed as the percentage of red cells haemolysed with reference to a standard. This standard was prepared for each test by adding 1 ml. of a 5 per cent suspension of red cells to 4 ml. of distilled water. After removing the

stromata by centrifugation, 4 ml. of buffer saline was added. A tube containing only the diluent and red cells was included in each test as a control on the stability of the red cells. Haemolysis that occurred during incubation period at 37°C (usually not more than 1 per cent) was deducted from the colorimeter readings of the preparations under test.

In cases where the colorimeter could not be used due to certain difficulties, the reading was taken visually and compared with the standard tubes (Nilakantan *et al.*, 1963). Results of the test carried out to compare the readings of the standard tubes with those obtained with the lumetron photoelectric colorimeter are given in Table I.

TABLE I. STANDARD TUBE READINGS (NILAKANTAN *ET AL.*, 1963) AND THE CORRESPONDING LUMETRON COLORIMETRIC READING IN HAEMOLYSIS OF FOWL CELLS BY NDV

Standard tube readings (per cent)	Colorimetric readings (per cent)
100	100
75	77
50	53
40	42
35	36
30	30
25	24
20	19·5
15	16
10	11
5	6

Preparation of lecithin and cholesterol suspensions: Lecithin and cholesterol were first dissolved in a small quantity of absolute alcohol and then diluted in buffer saline to the required concentration. The concentrations of alcohol used as primary solvent had no action either on red cells or on the virus.

(a) PRETREATMENT OF VIRUS WITH LIPIDS: In experiments where lipids were allowed to interact with the virus prior to the addition of the erythrocytes, serial dilutions of the virus were treated with a fixed concentration of these substances and the system was allowed to react overnight (20 hours) at 4°C and then the erythrocytes were added.

(b) PRETREATMENT OF FOWL ERYTHROCYTES WITH LIPIDS: Equal volumes of chicken red cells and suspension of the test compound were mixed thoroughly. The mixture incubated for three hours at 37°C. At the end of incubation period, the erythrocytes were washed three times in a buffer saline; 5 per cent suspension of the cells was used in the test.

(c) TREATMENT OF VIRUS-ABSORBED RED CELLS WITH THE LIPIDS: Virus in two-fold serial dilutions was allowed to interact with chick erythrocytes at 4°C in a refrigerator for three hours. At the end of this period the cells were washed thrice with cold buffer saline, resuspended in the required volume of lipid suspension and incubated at 37°C in a water bath for three hours.

RESULTS

Haemolysin titration using red cells from different fowls: To ascertain whether erythrocytes obtained from different fowls have uniform susceptibility to haemolysin by NDV, haemolytic activity of the R_2B strain was titrated using cells from 12 different birds. All the birds gave erythrocytes susceptible to haemolytic activity of the virus; however, the percentage of haemolysis varied in the case of individual birds from 22 to 40·5 per cent. The maximum percentages of haemolytic activity shown by individual birds were 22, 25, 27, 27, 27, 28, 28, 29, 31 and 40·5.

Haemolytic activity of untreated a.a. fluid virus: Six strains of NDV mentioned earlier were used for quantitative estimation of their haemolytic activity (Table II). With R_2B, Palestine and KB_{12} strains the highest percentage of haemolysis was obtained with 1: 20 dilution of the virus but not with the lower dilutions used in the test. These strains showed 20 to 25 per cent of haemolysis with fowl red cells. On the other hand, F and B_1 strains, which are of low virulence, showed only 7 to 8 per cent of haemolysis.

Haemolytic activity of frozen, thawed and dialysed a.a. fluid: The haemolytic activity of frozen, thawed and dialysed a.a. fluid and that of untreated a.a. fluid was titrated. The results are presented in Table II. The haemolytic activity of all the five strains

TABLE II. HAEMOLYTIC ACTIVITY OF UNTREATED AND FROZEN, THAWED AND DIALYSED ALLANTO-AMNIOTIC FLUID VIRUS SUSPENSIONS

Strains	Virus suspension	Virus dilutions							
		1:5	10	20	40	80	160	320	640
R_2B	Before treatment	17·0	22·0	28·0	27·0	18·0	12·0	7·0	4·5
	After treatment	35·0	30·0	25·0	20·0	15·0	10·0	5·0	3·0
Palestine	Before treatment	9·0	11·5	15·0	13·0	10·5	6·0	4·5	..
	After treatment	25·0	23·5	22·0	20·0	15·0	10·0	5·0	3·5
KB_{12}	Before treatment	15·0	18·5	25·0	23·0	19·0	14·0	9·0	4·0
	After treatment	45·0	40·0	30·0	25·0	15·0	10·0	7·0	..
B_1	Before treatment	8·0	7·0	6·0	4·5	3·0	..	..	..
	After treatment	60·0	50·0	40·0	30·0	25·0	20·0	10·0	4·0
F	Before treatment	7·0	5·0	4·0	3·0	..	..	..	..
	After treatment	85·0	80·0	70·0	55·0	40·0	30·0	20·0	15·0

Figures represent percentage haemolysis.

tested increased after the treatment, particularly so in the case of B_1 and F strains. The increase in the haemolytic activity in respect of R_2B, Palestine, KB_{12}, B_1 and F strains was 1·6, 1·6, 1·8, 7·5 and 12 times, respectively, as compared to the activity of untreated a.a. fluid. On no occasion the haemolysis was found to be 100 per cent.

Haemolysis of erythrocytes from different species of animals: The results are incorporated in Table III. The haemolysis produced by Palestine strain was weaker than that caused by the other strains. Further, all the strains haemolysed fowl, cattle, sheep, goat, guinea-pig, dog, duck and pigeon cells. Pig cells were haemolysed only by B_1 strain. Horse cells were weakly haemolysed by B_1 and F strains only. Although the strain KB_{12} agglutinated horse cells it did not cause lysis of the same. When untreated a.a. fluid was used in the test, the maximum percentage of haemolysis with avian cells was in 1: 20 dilution of the fluid in respect of all the strains under study; lower dilutions, viz., 1: 10 and 1: 5, produced haemolysis to a lesser degree. However, this effect was not observed with mammalian cells; the highest virus concentration produced maximum degree of haemolysis.

TABLE III. PERCENTAGE HAEMOLYSIS OF ERYTHROCYTES FROM DIFFERENT SPECIES OF ANIMALS BY NDV

Source of erythrocytes	Percentage of haemolysis				
	R_2B	Palestine	KB_{12}	B_1	F
Fowl	35	25	45	60	80
Bovine	35	20	30	45	30
Sheep	5	3	5	25	5
Goat	5	3	25	15	10
Horse	0	0	0	8	12
Guinea-pig	15	5	18	15	15
Rabbit	0	0	0	10	traces
Dog	15	5	20	15	10
Pig	0	0	0	5	0
Buffalo	5	0	15	22	6
Pigeon	15	traces	25	28	10
Duck	15	8	30	15	15
Rat	..	..	..	..	35

Effect of lecithin and cholesterol on the haemolytic activity of NDV: The results obtained are given in Table IV. As 0·05 mg./ml. of lecithin caused haemolysis of fowl erythrocytes, a lower concentration (0·025 mg./ml.) was used. Both lecithin and cholesterol inhibited to a certain degree the haemolytic activity of NDV. Pretreatment of the virus with both lecithin and cholesterol at 4°C for 20 hours inhibited to some

extent the haemolytic activity of the virus. However, when red blood cells were pretreated with the lipids at 37°C for three hours, only lecithin inhibited about 40 per cent of the virus activity, while cholesterol hardly inhibited 5 per cent of the virus activity. When the lipids were added after the virus cell interaction for a period of three hours in a refrigerator, hardly any inhibitory effect was noticed.

TABLE IV. EFFECT OF LECITHIN AND CHOLESTEROL ON THE HAEMOLYTIC ACTIVITY OF NDV

	Conditions of experiment	Lipids (mg. per ml.)		Percentage of haemolysis	Percentage inhibition
I	Addition of lipids just prior to addition of r.b.cs.	Cholesterol	0·0	70	
		,,	0·5	50	28·6
		Lecithin	0·0	50	
		,,	0·025	40	20·0
II	Pretreatment of virus for 20 hours at 4°C	Cholesterol	0·0	65	
		,,	0·5	50	23·0
		Lecithin	0·0	50	
		,,	0·025	35	30·0
III	Pretreatment of r.b.c. for three hours at 37°C	Cholesterol	0·0	58	
		,,	0·5	55	5·0
		Lecithin	0·0	50	
		,,	0·025	30	40·0
IV	Addition after virus-cell interaction for three hours at 4°C	Cholesterol	0·0	30	
		,,	0·5	28	6·7
		Lecithin	0·0	28	
		,,	0·025	27	3·6

DISCUSSION

The haemolysis caused by NDV was never complete. It stopped at certain stage of the process. This may perhaps be due to the destruction of the receptors of the cell by concomitant non-haemolytic moieties of the virus, as pointed out by Burnet and Lind (1950).

Although erythrocytes from all the 12 birds used in the study were haemolysed by NDV, there was a wide range of variation in the susceptibility of the cells from individual birds. The maximum amount of haemolysis produced by the same pool of virus with the cells from different birds varied from 22 to 40·5 per cent. This confirms the findings of Kahnke (1951).

With unheated a.a. fluid the maximum degree of haemolysis of fowl cells was produced by 1 : 20 dilution of the virus but not by lower dilutions; this indicates the presence of some inhibitor in the a.a. fluid which influenced the activity of the virus in lower dilutions, as indicated by Chu and Morgan (1950b) and Morgan (1951). However, the behaviour of B_1 and F strains was different in that the lowest dilution of virus produced highest amount of haemolysis.

There were minor but distinct differences in the range of haemolytic activity of different types of avian and mammalian cells by the five strains of NDV. This may be due to differences in their enzymatic make-up in this respect, and are perhaps reflected on the antigenic properties of the strains.

In the case of mammalian cells the percentage of haemolysis was proportional to the concentration of the untreated virus. It is probable that there may be fundamental differences in the mode of action of the inhibitor with respect of two types of cells. The results of experiments on the effect of freezing, thawing and dialysis on the haemolytic activity of a.a. fluid virus were in conformity with the findings of Burnet and Lind (1950) and Morgan (1951).

Freezing, thawing and dialysis also brought to surface emphatic differences with respect to five strains of NDV. Low virulent strains like B_1 and F showed a pronounced activity, which was far in excess of that shown by the strains of somewhat higher virulence, such as Palestine, R_2B and KB_{12}, as judged by their embryo infectivity. It is presumed that in the case of the virulent strains certain inhibitors may be liberated in the tissues of the chick embryo after death, and that these are perhaps not removed by freezing, thawing and dialysis.

It would seem that haemolysis reaction could be relied upon for evaluating the quantity of virus content in the ND vaccine in the place of the conventional procedure embracing active immunization of fowls and challenge.

As reported earlier by Morimoto and Morgan (1954) with mumps virus, the present study revealed that lecithin and cholesterol in low concentrations caused a fall in the haemolytic activity of NDV. The retardation of haemolytic activity of NDV was not marked when the substances were allowed to come in contact with the virus just before the addition of erythrocytes. There was, however, a striking difference in the activity of the two substances when pretreatment of the erythrocytes was carried out, lecithin alone rendering them less susceptible to haemolysis. The selective inhibition action of lecithin might suggest that the virus is perhaps equipped with an enzyme system which may bring about dissolution of the lipid on the cell surface and that this effect is partly counteracted by the free lecithin added to the virus so that its specific action on the erythrocyte is minimized. The possibility of the mumps virus haemolysin acting on the phospholipid component of the red cell surface has been indicated by Moberly *et al.* (1958).

SUMMARY

Haemolytic activity of six strains of NDV of different grades of virulence on erythrocytes of different species of animals and birds was studied. Haemolysis produced by the infected untreated a.a. fluid was comparatively weak. In the case of avian cells

while the lower dilution of the virus produced lesser degree of haemolysis, a 1:20 dilution produced maximum haemolysis.

Haemolysis produced by NDV was found to be never complete. The range varied from 22 to 44 per cent when erythrocytes from 12 different fowls were used for the test.

Freezing, thawing and dialysis of the a.a. fluid preparations appeared to have a salutary effect on the virus haemolysis activity, although differences among strains were manifested.

There were marked differences in the range and percentage of haemolysis of erythrocyte suspensions from different mammalian and avian species by the five strains of NDV. While haemagglutination was a preliminary stage in the haemolysis reaction, it was noted that in all cases the haemagglutination reaction was not followed by haemolysis. The KB_{12} strain of the virus agglutinated but did not cause any haemolysis of horse redblood cells.

Lecithin and cholesterol were found to inhibit partially the haemolytic effect of the virus. The inhibiting action was, however, apparent only when the union of the virus with red cells had not taken place prior to the addition of the lipids. Pretreatment of red cells with lecithin rendered the cells less susceptible to the action of virus haemolysin, but cholesterol did not have any significant inhibitory effect under similar conditions.

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ABSTRACTS

Effect of nutrient density and protein-energy inter-relationships on reproductive performance of the hen. TOUCHBURN, S. P. and NABER, E. C. 1962. *Poult. Sci.* **41:** 1481-88.

The term nutrient density was introduced during the present study to describe the concentration of known nutrients in relation to the total weight of ration. The nutrient density of rations fed to Leghorn laying-pullets was varied from 16 per cent protein and 960 calories of productive energy per pound at a calorie-protein ratio of 60. In all rations the animal protein, vitamins and minerals were provided in proportion to the total protein. The rate of egg production was not significantly affected by the nutrient density of the ration. A calorie-protein ratio of 80 in a 12 per cent protein ration caused a decrease in egg production. The efficiency of feed utilization increased as the nutrient density (concentration of known nutrients per pound) increased. The reduction in relative efficiency with the highest nutrient density ration (20 per cent protein, 1,200 cal./lb. productive energy), was attributed in large measure to over-consumption. It was observed that the energy and protein levels in the laying ration should be adjusted to permit a daily intake of at least 17 gm. of protein per bird for maintenance of 72 per cent egg production.

In all experiments fertility and hatchability were maintained at an exceptionally high level. The average fertility was 94 per cent and the average hatchability was 90 per cent. These characteristics provide very sensitive criteria of the nutritive adequacy of the experimental diets, especially with regard to vitamin and mineral content.—(S. B.)

Relationship between hog-cholera virus and diarrhoea virus of cattle. SHEFFY, B.E., COGGINS, L. and BAKER, J. A. 1962. *Proc. Soc. exp. biol. Med.* **109:** 349-52.

The authors have presented the concept of resistance or protection having no direct immunological basis by the use of cattle virus diarrhoea (VD), virus against hog-cholera (HC) virus in the same way as Jenner used cow-pox virus vaccine to protect human beings against small-pox virus, immunologically related to each other.

In their repeated three sets of experiments using lots of litters of pigs and calves against two strains of VD virus—Oregon C, 24V and New York 1 and one strain of HC virus—serum samples were collected before and after infecting or inoculating with the respective strain of virus and after challenge at 4, 7, 10, 14 and 21 days.

By serum neutralization tests with the tissue culture antigens it was observed that pigs that were initially given a dose of VD virus, though revealed before challenge a low antivirus diarrhoea titre of 27 at 28 days, showed an anti-hog-cholera titre of 515 at 21 days after challenge with HC virus. The titre against VD also rose from 27 to 729.

In the reciprocal tests calves initially inoculated with HC virus showed a low titre of HC antibodies; but it rose to a peak parallel to VD antibody titre of 988 at 21 days, starting from the fifth day after challenge with the virulent VD virus.

Serum samples from calves that had not been given HC virus showed VD antibodies from the 11th day after inoculation with VD virus and rose to a titre of 720 at 21 days stage.

From this the authors have concluded that: (1) VD virus protects pigs against a lethal dose of HC virus; (2) the two viruses failed to share a common neutralizing antibody; (3) the VD virus appears to create a stage of secondary response to HC virus, which is otherwise fatal to them; this stage resembles the effect induced by a single inoculation of homologous inactivated viral antigen; and (4) the HC virus, though apparently failed to give absolute solid immunity against VD virus, definitely indicated the effect of secondary response.—(M. N.)

Liveability of bovine spermatozoa at 5°C in Tris-buffered and citrate buffered yolk-glycerol extenders. DAVIS, I. S., BRATTON, R.W. and FOOTE, R. H. 1963. *J. Dairy Sci.* **46** : 57-60.

Bovine spermatozoa could be better preserved at 5°C in 0·2 M Tris-buffered yolk-glycerol extender (TYG) than either in CUE or in citrate-yolk-glycerol extender (CYG) used routinely for freezing bovine semen. After eight days of storage at 5°C, the average motility in TYG was 42 per cent whereas in CUE and CYG it was 35 and 18 per cent respectively. The composition of Tris-buffer is: Tris (Hydroxymethyl) aminomethane (3·0284 gm.), citiric acid (1·6918 gm.), glycerol (14·0 gm.) (This is determined by using specific gravity of glycerol 1·25) and glass redistilled water 100 ml.

The pH of the buffer is 6·75. While using, 80 per cent of the buffer should be mixed with 20 per cent of egg-yolk and fortified by 500 units of penicillin and 500 μg. of dihydrostreptomycin per millilitre of the extender.—(D.P.M.)

Vaccination of dairy cattle against staphylococcic mastitis. BLOBEL HANS and BERMAN DAVID, T. 1962. *Amer. J. vet . Res.* **23** : 7-14.

A bacterim-*cum*-toxoid was prepared to which measured amounts of coagulase and later egg-yolk factor were added for vaccination of dairy cattle against staphylococcic mastitis. A toxigenic strain of *Staphylococcus aureus* isolated from a cow with acute mastitis was used for the preparation of the vaccine. The strain belonged to the phage type 52, 55, 42D, and produced alpha, beta and delta haemolysins. The organism was grown in brain heart-infusion broth on a rotary shaker for three days at 37°C. At this time the culture contained approximately 2×10^9 cells per ml. and maximum amounts of haemolysins. The coagulase isolated from the vaccine strain was concentrated by acid precipitation followed by two cycles of ethanol fractionation. Inactivation of staphylococci and detoxification were accomplished by addition of formaldehyde at a final concentration of 0·4 per cent and incubation of the mixture for three to four weeks. Potassium aluminium sulphate was added to a final concentration of 0·5 per cent.

A group of 18 dairy cattle composed mainly of the Holstein-Friesians were vaccinated; first three injections of 6 ml. of vaccine given intramuscularly at two week intervals were followed by four booster injections of 10 ml. each three to six months apart. Approximately six weeks after the first vaccination the 18 vaccinated and 18 non-vaccinated cows were exposed to staphylococcal mastitis by introducing into the herd four virulent *Staph. aureus* strains, one vaccine and three others which could be differentiated from each other by laboratory tests. It was observed that vaccinated cows were more resistant to mammary infections with the homologous than those with heterologous staphylococcic strains. Vaccination also resulted in some degree of protection against infections caused by heterologous strains. Infections in vaccinated animals were rather mild and characterised by the presence of fibrin clots and increased numbers of leucocytes in milk without signs of a generalised disease. Staphylococcic mastitis in non-vaccinated cows, however, was generally more severe. It was associated with fever and inappetance and persisted longer.

The specific effects of vaccination could not be related to any single antibody—(V.K.Y.)

The effect of progesterone and progestational compounds upon the production of laying pullets. COOK, R. E. and WARNICK, A. C., 1962. *Poult. Sci.* **41**: (**5**): 1545-49.

In two separate experiments the effect of administration of crystalline progesterone or a progestational compound Provera (6-methy-17 acetoxy progesterone) by different methods and in different dosage on egg laying (production) of commercial White Leghorn type pullets was studied. During the laying period all pullets received a 17 per cent protein diet that had 940 calories of productive energy per pound.

During the first experiment, production was recorded for 28-day pre-treatment, 15-day treatment and 28-day post-treatment or recovery periods; crystalline progesterone in quantities of 0, 2, 4, 8, 16, 32 and 64 mg. in total, dissolved in corn oil was injected subcutaneously in the birds either in a single dose or in five divided doses at three-day interval.

In the second experiment Provera was administered either in gelatin capsules every third day in five oral doses making total quantities of 0, 4, 8, 16, 32 , 64 and 128 mg. or in the form of 20 mg. pellets placed under the skin in dosages of 6, 20, 40, 80 and 160 mg. Production records were maintained over 5-week pre-treatment, 15-day treatment and 28-day post-treatment and a 17-week recovery laying periods.

Crystalline progesterone caused a significant decline in production during the treatment and the following 28-day post-treatment periods. This suggests that the production of gonadotropic hormone was reduced or inhibited by the progesterone. No such effect was observed following the administration of Provera, as the dosage levels were not high enough to cause an inhibition of gonadotrophic hormones in the pullets.

Pullets ranked in different groups on the basis of pre-treatment production levels retained the same relative ranks following treatment and during the post-treatment period.—(C.M.S.G.)

REVIEW

CROSSBREEDING BEEF CATTLE. Edited by CUNHA, T. J., KOGER, M. and WARNICK, A. C. 1963. University of Florida Press, 15 N. W. 15th Street, Gainesville, Florida. pp. viii + 228; Figs. 47; Price 8·50.

Breed formation is a continuous process all through the recorded history of animal husbandry. However, it is only for a few decades that breeding is being conducted in a systematic way to meet the needs of a given geographic, climatic or economic situation. *Crossbreeding Beef Cattle* is a compilation of reports submitted by the Department of Animal Science, University of Florida, in 1961.

It provides information on beef cattle of the various parts of the world with reference to their breeds, geographical distribution, factors responsible for their distribution, points of their origin, classification, etc., along with charts and figures. This information is very useful and interesting. Formation of new types, factors involved in forming new breeds, their performance, the reasons for developing them, and the success man has achieved in forming them have been discussed. A few strains evolved by man are also described.

In a number of chapters breeding experiments carried out in various parts of the world, primarily in the U.S.A., are presented in detail. The practical system of breeding described in these chapters can be suitably modified for adoption.

For successful crossbreeding it is very essential for the breeder to have a good knowledge of adaptability of adopted animals and crossbreeds, climatic conditions, blood composition and physiology, performance of crossbreeds, etc. Besides, one would like to know the advantages and disadvantages of crossbreeding. A few chapters have been devoted to explain these factors.

Hybrid Vigour and Feed Conversion, Mothering Ability, Hybrid Calf Fattening and Feedlot Performance of Crossbreeds are interesting chapters. The last chapter 'Practical Crossbreeding Plans' is of great practical utility.

The information given in this publication is valuable. The editors deserve compliments for putting forth this topic in such a simple and lucid style. This book is a must for veterinarians, students and research workers alike. The book consists of 47 valuable illustrations, which add to the information and beauty of the publication. The getup is excellent.—(R.R.L.)

EFFECT OF SOME CLIMATOLOGICAL FACTORS ON REPRODUCTION OF BUFFALOES (*BUBALUS BUBALIS*)

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A VARIETY of breeds of domesticated animals with general and specific adaptability to the varied conditions in which they are reared are found in India. Although the animals can survive varying environmental conditions, they fail at times to combat the factors responsible for changed physiological status, affecting their production and reproduction. Goswami and Prem Narain (1962) reported significant variation in the rate of respiration and body temperature of buffaloes (*Bubalus bubalis*) due to variation in the ambient temperature. They found maximum influence on their physiological norms, when air temperature and relative humidity are taken together. The variation reported is highly significant and it may be affecting the normal physiology of reproduction of the species. That high environmental temperature affects the fertility of cows adversely was reported by Villegas (1939), Hammond (1949), Bonsma (1949) and Bonsma *et al.* (1953). Ulberg (1958) also concluded that the adverse effect of high environmental temperature may either be on the ova or on the uterine environment of the females. Goswami *et al.* (1961) reported seasonality in the calving of buffaloes using 12,146 observations from different farms in the country. Similar observations were reported by Ramanathan (1932), Dave (1938), Rao and Murari (1956), and Pattabiraman (1956) in Indian water buffaloes. The seasonality of calving of buffaloes is largely responsible for more supply of milk during winter and considerably low supply of milk during summer. Since genetic selection of animals calving during 'off season' for ensuring even supply of milk throughout the year has not been found to be a practical proposition (Goswami *et al.*, 1961), the present study was undertaken to find out the effect of bad climate and possibilities of extending favourable environment to the animals, which may help in the solution of the problem of spreading out the breeding/calving season in buffaloes. Brezowsky and Haeger (1959) reported that favourable weather leads to more cows coming in season, and poor weather leads to a reduced onset of heat. Dale *et al.* (1958) reported that it is possible to obtain early maturity at certain range of temperature and relative humidity in certain breeds of cattle. So far as the physiological and reproductive changes or rhythms of animals due to climatic factors are concerned, they may be due to the influence of air temperature and relative humidity. The present study is on the effect of air temperature and relative humidity and their extent, independently and in combination, on conceptions and calvings in buffaloes.

MATERIAL AND METHODS

Data on various aspects of reproduction of buffaloes were collected from the pedigree sheets from different farms in the country (Goswami *et al.*, 1961). To study

the effect of air temperature and relative humidity on calvings and conceptions of buffaloes, five years' data relating to these factors were considered. The meteorological data were collected from the daily records of the Indian Weather Report of the Government of India, for the respective stations of the buffalo-breeding farms (Table I). The data for calvings pertain to 1954-1958 and that for conception 1953–1957, except for the Aarey Milk Colony, Bombay. For this Farm the respective years are 1956–1960 and 1957–1959. The managerial conditions at the farms are taken as more or less uniform throughout the year.

TABLE I. INFORMATION ON THE GEOGRAPHICAL LOCATION OF THE FARMS

Farm	Latitude °N	Longitude °E	Rainfall annual (Normal in cm.)
Military Farm, Bangalore	12·58	77·38	87
Military Farm, Secunderabad	17·27	78·33	75
Government Dairy, Vishakhapatnam	17·42	83·20	96
Aarey Milk Colony, Bombay	18·55	72·54	181
Military Farm, Jabalpur	23·10	79·59	146

The statistical methods adopted for the analysis of the data are as per Snedecor (1956).

RESULTS

The monthly percentage of the basic data on conception and calving of buffaloes are presented in Table II. The data indicate that there is month-to-month variation in the variables. The chi-squares (χ^2) based on a number of observations show that the rate of conception and calving of buffaloes vary between and within the farms. While the maximum air temperature is attained mostly either during April or May or June at the farms, the minimum is found either during December or January. There is an appreciable range between minimum and maximum air temperature and relative humidity at certain farms. The relative humidity is highest during August for most of the farms (for Jabalpur it is during July) but for minimum the months vary. The climatic data also indicate that the range for relative humidity is narrow for coastal farms, viz., Vishakhapatnam and Bombay as compared to the rest of the farms. Jabalpur Farm presents the data for extreme climate, both for air temperature and relative humidity.

Effect of air temperature and relative humidity on conception of buffaloes: Since the data on conception of buffaloes and the climatic factors range over the period of five years, it was essential to eliminate heterogeneity arising due to intermediate years. The data were therefore subjected to analysis of variance and co-variance, and partial regression equation obtained from the residual sum of squares and products. The partial correlation co-efficients, whereby the effect of one variable over the other keeping the rest constant is determined, were estimated.

TABLE II. TREND OF CONCEPTION AND CALVING OF BUFFALOES IN DIFFERENT MONTHS

Farm	Per cent	Months												Total No. of observations	χ^2
		Jan.	Feb.	March	April	May	June	July	August	Sept.	Oct.	Nov.	Dec.		
Military Farm, Bangalore	Conception	13·28	9·28	8·64	5·37	3·05	1·96	1·50	2·55	6·23	12·60	18·61	16·93	2,198	1,015·74**
	Calving	8·84	5·69	3·57	1·91	1·37	1·99	6·48	10·47	19·35	17·86	12·50	9·97	2,408	1,155·93**
Military Farm, Secunderabad	Conception	11·52	6·61	5·58	4·95	4·91	3·87	3·83	3·38	6·30	14·85	19·62	14·58	2,222	858·14**
	Calving	6·44	4·53	5·33	3·82	3·10	2·74	4·30	13·60	20·40	15·43	10·89	9·42	2,515	1,067·60**
Govt. Dairy, Vishakhapatnam	Conception	11·12	8·45	7·17	3·10	3·21	3·21	5·45	8·13	6·74	10·91	15·61	16·90	935	261·47**
	Calving	7·33	5·13	3·99	4·05	4·34	7·90	7·55	11·46	13·52	13·31	10·39	11·03	1,405	233·08**
Aarey Milk Colony, Bombay	Conception	14·47	9·38	8·25	4·53	3·15	2·42	2·42	5·90	8·65	13·58	12·05	15·20	1,237	362·77**
	Calving	8·08	4·55	3·53	2·61	1·89	5·52	7·55	14·04	13·75	14·37	14·09	10·02	2,066	631·01**
Military Farm, Jabalpur	Conception	8·83	8·42	5·91	3·72	2·98	3·12	2·46	4·60	12·14	21·12	14·42	12·28	2,150	934·49**
	Calving	5·97	4·05	2·61	2·66	3·09	3·57	10·19	20·86	15·03	12·28	9·54	10·15	2,296	1,004·58**
Pooled data	Conception	11·68	8·32	6·98	4·49	3·53	2·93	2·88	4·34	8·10	15·22	16·59	14·94	8,742	2,989·83**
	Calving	7·32	4·77	3·83	2·94	2·64	3·97	7·11	14·26	16·82	14·82	11·52	10·00	10,690	3,570·16**

**Significant at 1 per cent level.

The summary of the statistical analysis of the data on conception of buffaloes and the climatological factors is presented in Table III.

TABLE III. SUMMARY OF THE STATISTICAL ANALYSIS OF CONCEPTION OF BUFFALOES AND THE CLIMATIC FACTORS

Farm	Partial correlations		Multiple correlations	Variation (per cent)
	Air temperature (°C)	Relative humidity (per cent)		
Military Farm, Bangalore	—0·610**	—0·361**	0·616**	38·21
Military Farm, Secunderabad	—0·726**	—0·396**	0·726**	52·83
Government Dairy, Vishakhapatnam	—0·653**	—0·074	0·692**	48·02
Aarey Milk Colony, Bombay	—0·281**	—0·052	0·279	7·81
Military Farm, Jabalpur	—0·501**	+0·008	0·536**	28·75

** Significant at 1 per cent level.

The multiple correlations estimated for most of the farms are significant except for Bombay. The influence on conception due to climatic factors is also marked. It is interesting to note that the Bombay Farm, as seen from the multiple correlation, does not show significant effect on this phenomenon and as such the data are subjected to further analysis to find out probable reason for such an effect.

Table III also shows that the effect of air temperature on conception of buffaloes, when relative humidity is held constant, is negative for all the farms. It is also highly significant. This indicates that as the air temperature increases the number of conception decreases and as the air temperature decreases the number of conception increases.

When air temperature is held constant the effect of relative humidity shows a general negative trend for all the farms except the Jabalpur Farm. It shows that with the decrease of relative humidity the number of conceptions increase, whereas with the increase in relative humidity the rate of conception decreases. The influence of relative humidity is significant for Bangalore and Secunderabad farms.

The variation percentage (Table III) estimated on the basis of the multiple correlations for the farms show that there is a large variation from farm to farm. This indicates varying degree of influence of the climatic factors on conception of buffaloes at different farms.

Curvilinearity of regression of air temperature on conception of buffaloes at Bombay Farm was tested and was found to be non-significant. It indicates that the multiple correlation presented in Table III is dependable and the partial correlation for air temperature and relative humidity estimated show the same trend as in case of the other farms.

Effect of air temperature and relative humidity on calving of buffaloes: The data on calvings of buffaloes and the climatic factors for different farms were subjected to analysis on the lines indicated earlier. The summary of results is presented in Table IV.

TABLE IV. SUMMARY OF THE STATISTICAL ANALYSIS OF CALVING OF BUFFALOES AND THE CLIMATIC FACTORS

Farm	Partial correlations		Multiple correlations	Variation (per cent)
	Air temperature (°C)	Relative humidity (per cent)		
Military Farm, Bangalore	—0·396**	+0·317**	0·557**	31
Military Farm, Secunderabad	—0·232	+0·476**	0·583**	34
Government Dairy, Vishakhapatnam	—0·094	+0·451**	0·457**	21
Aarey Milk Colony, Bombay	—0·129	+0·616**	0·619**	38
Military Farm, Jabalpur	—0·555**	+0·843**	0·842**	71

** Significant at 1 per cent level.

Table IV indicates that the multiple correlations estimated for different farms to determine the effect of climatological factors on calving are highly significant, and that climate influences significantly the reproductive phenomenon of calving of buffaloes. The relative humdity is held constant, the air temperature has a negative correlation with the characteristic of calving of buffaloes. It means there is an increase in the trend of calving of buffaloes with decreasing air temperature and *vice versa*. Similar trend is shown for all the farms and the effect is highly significant for the Bangalore and Jabalpur farms.

When air temperature is held constant, the influence of relative humidity on the characteristic is positive, which means more the relative humidity, more is the number of calvings and *vice versa*. The effect of relative humidity is shown significantly high for all the farms.

The percentage variation as explained by the multiple regression equation varies from farm to farm (Table IV). It means that the climatic factors have varying degree of influence on calving of buffaloes from farm to farm.

DISCUSSION

From the results presented, it is evident that more conceptions in buffaloes occur during the period when both the environmental air temperature and relative humidity are less. It is also observed that high environmental temperature affects adversely the conception, i.e., fertility in buffaloes. These findings agree with that of Villages (1939), Hamond (1949), Bonsma (1949) and Bonsma *et al.* (1953) on cows.

The period of low air temperature and relative humidity in India is the one generally referred to as winter, during which buffalo-bulls produce good-quality semen (Malkani, 1954; Kushwaha *et al.*, 1955). It seems coincidental that higher rate of conception in buffalo-cows should occur during that season. It is also important to note that during this period the breeding efficiency of buffalo-bulls as well as buffalo-cows is at the peak. This indicates that the normal reproductive activities of spermatogenesis in buffalo-bulls and oogenesis in buffalo-cows are simultaneous.

The present study indicates that the conceptions in buffalo-cows are less during the period of high environmental temperature and that it further decreases as the relative humidity increases in the environment. During the period of high environmental temperature and relative humidity the semen quality of buffalo-bulls is also at the lowest ebb. Therefore, the low conception in buffaloes during this period may, first, be attributed to bad quality of buffalo-bull semen, which may be due to hypothyroid activity (Bhatnagar *et al.*, 1955)or, secondly, from the present study it may be deduced that in buffalo-cows also there may be hypoactivity of the thyroid gland, which is responsible for this low rate of conception. The buffalo-cows are in the late stage of pregnancy during this period and tend to calve when more of air temperature coupled with more of relative humidity is present in the environment, as can be seen from the findings of this study. It is important to note that such environment should be conducive to the calving of buffaloes as against the same environment being unhelpful to the production of good-quality semen. The present study indicates that there are maximum number of calvings during this period of the year. The principal hormones directly concerned with pregnancy and the process of calving are the pituitary hormones other than follicle-stimulating hormone (F.S.H.) and luteinising hormone (L.H.). The F.S.H. and L.H. are responsible for spermatogenesis in males and oogenesis in females. The present study indicates that F.S.H. and L.H. are not normally secreted during this period. As a result of this the semen quality of buffalo-bulls may be bad and the buffalo-cows do not appear in normal oestrus, resulting in less number of conceptions.

When the level of progesterone is high, the secretion of F.S.H. and L.H. by the pituitary gland is retarded. And, as the function of the thyroid gland in reproduction is presumed to be concurrent with the secretion of F.S.H. and L.H., as a result of retardation of these hormones the thyroxine secretion of the thyroid gland is also retarded. It is probably for this reason that there is a natural provision that after the normal function of the progesterone to maintain pregnancy and of the posterior pituitary to secrete oxytocin hormone for calving, the anterior pituitary is activated to secrete F.S.H. and L.H., which are inducive to oestrus and conception. Thus, after the period of maximum calving—which comprises high air temperature coupled with high relative humidity—the favourable period for fertility is followed. This period, as described earlier, comprises low air temperature in association with low relative humidity. The period is favourable for production of good-quality semen besides a large number of buffalo-cows appear in oestrus which may be seen from the rate of their conception during these months. It may be observed from the findings of this study in case of buffaloes that the environment which is good for calving is bad for conception and it appears to be a natural phenomenon. It may also be observed that the trend of endocrine secretion from the anterior pituitary of buffalo-bulls for spermatogenesis as well as of buffalo-cows for oogenesis is identical as nature's provision for successful mating.

It may be deduced as a result of the present study that cool (low) air temperature with low relative humidity is inducive to the conception of buffaloes (for good-quality semen production of buffalo-bulls also); such an artificial condition to the animals during the period of high environmental temperature may prove helpful in inducing the animals to come in oestrus, which on service/insemination would cause

conception. This may contribute to space out the breeding/calving season in buffaloes to ensure an even supply of milk throughout the year.

SUMMARY

The study was conducted on a total number of 8,742 observations for conception and 10,690 for calving of buffaloes obtained from five buffalo-breeding farms located at different geographical positions in the country. The climatic data on air temperature and relative humidity were considered for the corresponding five years on the basis of the daily records for the respective stations published by the Meteorological Observatory of the Government of India. The study revealed the following facts :

1. There is a marked effect of the climatological factors on conception and calving of buffaloes.
2. The degree of influence of air temperature and relative humidity on conception and calving of buffaloes vary from farm to farm.
3. The number of conceptions in buffaloes increases with the decrease in air temperature and *vice versa*.
4. There is an increase in conception of buffaloes with the fall in relative humidity at lower air temperature and *vice versa*.
5. There is a high number of calvings when atmospheric temperature is high, coupled with high percentage of relative humidity and *vice versa*.
6. Hypothyroid activity in buffalo-cows is also evident during the period when high air temperature in association with high relative humidity is present in the environment.
7. It is observed as a result of this study that cool (low) air temperature with low relative humidity being inducive to conception of buffaloes (for good quality semen of buffalo-bulls also); such an artificial condition to the animals may help solve the problem of spreading out the breeding/calving season in buffaloes to ensure even supply of milk throughout the year.

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STUDIES ON SURRA

IV. ACTION OF BERENIL, SURAMIN AND QUINPYRAMINE ON *TRYPANOSOMA EVANSI*

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EXPOSURE of *Trypanosoma equiperdum* to antrycide *in vitro* (Ormerod, 1951; Hawking and Thurston, 1955) and *in vivo* (Ormerod, 1951) has been reported to impair its infectivity to subinoculated mice. This paper deals with the effect of berenil, suramin and quinpyramine on the infectivity of *T. evansi*, changes caused by the treatment in morphology of the trypanosome and on the mode of the trypanocidal action of these drugs.

MATERIAL AND METHODS

Adult albino rats were infected subcutaneously with 1 ml. of blood (of a rat showing teeming parasites in the tail blood) diluted 1:10 with citrate-saline. When showing intense parasitaemia they were treated with an intraperitoneal inoculation of 0·5–1 ml. of aqueous solution of trypanocide. Tail blood from the treated rats was diluted 1:40 with citrate-saline, and 0·2 ml. of this solution was subinoculated intraperitoneally into each mouse. Subinoculations were carried out within few minutes of their harvesting from the donor rats. The inoculum, when examined under microscope, contained a variable number of trypanosomes. In the experiments on subcurative doses of suramin and quinpyramine, trypanosomes were counted in the inoculum. Tail blood of subinoculated mice was examined on every alternate day for appearance of trypanosomes for 30 days. If within this period trypanosomes failed to appear the subinoculum was considered as non-infective. The two strains of *T. evansi* used were the Izatnagar strain (Gill, 1961) and the Madras strain (Gill and Sen, 1963). Rats Nos. 400 and 401, 405 and 407, and 14 X and XX employed in experiments with berenil, suramin and antrycide, respectively, were infected with the Izatnagar strain; the remaining rats were infected with the Madras strain (Tables I, II, III). Thus, each drug was tried on two strains. Both were virulent strains and killed rats and mice within five and four days respectively.

As the results of the first series of trial with each drug at curative dose rate employing only one strain did not confirm *in toto* the published work (Ormerod, 1951; Hawking and Thurston, 1955), the experiments were repeated two to four times in series using two different strains; the subcurative dosage of suramin and quinpyramine was also employed to judge the reproducibility of the conclusions.

Two blood smears prepared at each subinoculation were dried in air and stained with Giemsa after fixation in acetone-free methyl alcohol. They were examined for

appearance of trypanosomes under oil-immersion lens of a Zeiss binocular research microscope using the artificial light corrected through blue filter provided for the purpose.

Parasitaemia was judged using tail blood by one of the following methods : (1) by an arbitrary scheme mentioned under Results, or (2) by counting the trypanosomes with a haemocytometer using the following diluting fluid : 1 per cent methylene blue 2 ml., glacial acetic acid 0·25 ml. and water 50 ml. (Hawking and Sen, 1960). The drugs employed were berenil (4-4′diamidino-diazoamino benzene; Hoechst), suramin (antrypol, I.C.I.) and quinpyramine (antrycide methyl sulphate; I.C.I.). Solutions were prepared fresh in double-distilled water off Pyrex glass.

For taking tail blood a separate pin was used for each mouse/rat.

RESULTS

Infectivity of blood of rats after treatment with trypanocides : The data on infectivity are given in Tables I, II and III.

TABLE I. INFECTIVITY OF THE PERIPHERAL BLOOD OF RATS AFTER TREATMENT WITH QUINPYRAMINE AT 4 MG./KG. (SERIES 1-4) AND AT 1 MG./KG. BODY WEIGHT (SERIES 5)

S.	Rat No.	O hr.	1 hr.	4 hr.	8 hr.	24 hr.	36 hr.	48 hr.	54 hr.	60 hr.	70 hr.	80 hr.
1	101 P*	370		350	400	30	N	N				
	I**			4/6	1/3	4/4	2/4	1/5				
				(3-19)	(20)	(9-11)	(11-21)	(17)				
	102 P	580		680	500	N	N					
	I			2/4	2/3	3/3	0/5					
				(6)	(6)	(6)						
	14 P	T	212	187	180	R	R	R		N		
	I	8/8	8/8	8/8	8/8	8/8	8/8	6/6		6/6		
		(1-2)	(2-7)	(4-10)	(7-18)	(6-14)	(7-19)	(11-15)		(13-20)		
2	X P	T	400	440		R	R		R	N		
	I	8/8	8/8	8/8		8/8	4/4		4/4			
		(1-3)	(6-15)	(5-14)		(9-16)	(6-19)		(3-15)			
	XX P	T	380	400		R	R		N			
	I	5/5	4/6	6/7		8/8	6/6					
		(1-4)	(1-10)	(6-18)		(7-14)	(7-13)					
3	201 P			398	180	N						
	I			8/8	7/7	6/8						
	109 P	230		190		100	70	50	30	N		
	I							1/4	1/4	0/4		
								(16)	(14)			
	110 P	200			240	170	50		30	N		
							1/2			1/7		
							(11)			(23)		
	111 P	190			240	140	50	60	48	N		
	I						1/4	1/5	1/6	1/4		
							(11)	(13)	(26)	(11)		
4	55 P	T			460	100	N	N		N		
	I				6/6[27]	5/5[5]		0/3		0/1		
					(7)	(6)						

TABLE I—(*Concld.*)

S.	Rat No.		0 hr.	1 hr.	4 hr.	8 hr.	24 hr.	36 hr.	48 hr.	54 hr.	60 hr.	70 hr	80 hr.
	56	P	T			800	300	300	N		N		
		I				6/6[44] (4-7)	4/4[15] (4-5)	4/4[15]	0/5		0/4		
	57	P	+++			300	120	20	N		N		
		I				6/6[15] (5-7)	5/5[6] (4-6)	4/4[1] (5-6)	6/6 (7-10)		3/3 (10-14)		
5	61	P	370		500	270	220	180	20		R	R	N
		I			6/6[25] (4-6)	6/6[13] (4-6)	7/7[11] (5)	8/8[9] (5-7)	8/8[1] (4-6)		7/7 (4-5)	6/6 (6)	6/6 (6)
	62	P	400		500	520	720	600	100		60	40	5
		I			6/6[25] (4)	6/6[26] (4)	6/6[36] (5)	8/8[30] (5)	8/8[5] (4-6)		6/6[3] (4)	8/8[2] (4-6)	6/6[25] (6)

*Parasitaemia expressed either as thousands of trypanosomes per cm. of the blood, or classified arbitrarily as follows:

T=Teeming parasites as seen under high power of microscope.
+++=11-20 trypanosomes/field.
+=1-5 trypanosomes/field.
R=Fewer than 1 trypanosome/field.
N=No parasites seen.

**Infectivity expressed as the number of mice infected/the number of mice injected.
Figures within (....) represent days within which the infected mice developed the infection.
Figures within [....] represent thousands of trypanosomes in the inoculum given to each mouse.

WITH QUINPYRAMINE : In rats Nos. 14 and X treated at 4 mg./kg. (Fig. 1) and rest of the rats treated at 1 mg./kg. the infectivity was not affected at all as blood was infective to all the subinoculated mice as long as trypanosomes were detected in the tail blood of the donors (Table I). In rest of the treated rats the infectivity was lost for

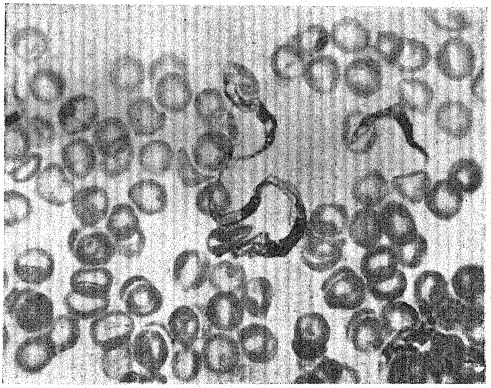

FIG. 1. BLOOD SMEAR OF RAT NO. 14 TREATED WITH QUINPYRAMINE AT 4 MG./KG. BODY WEIGHT, SHOWING 'INCLUSION BODIES' IN THEIR CYTOPLASM

some of the subinoculated mice. At no time was it completely lost as long as trypanosomes were detectable with microscope, and even when trypanosomes were not seen in blood the latter proved infective to varying proportion of the subinoculated mice. In cases where infectivity was lost for a proportion of mice, its loss was not progressive but erratic as in rat No. 101. The infectivity was 1/3 at the eighth hour but 1/4 at the 24th hour, though toward the end of the infection the infectivity was generally observed to be the least. It was also observed that the prepatent period usually became longer as the infection moved away from the treatment (0 hr.), except in the case of rats Nos. 61 and 62 treated at 1 mg./kg. when it remained almost of the same duration at various intervals after treatment.

The observations regarding 1 mg./kg. dose rate are comparable to that of Ormerod (1951), who also found that the drug at 2 mg./kg. injected in mouse did not abolish the infectivity of *T. equiperdum* infection. Ormerod (1951) observed that 5 mg./kg. dose rate abolished the infectivity of the rat infection after 24 hours, though trypanosomes persisted in the blood for several days; however, in the present case at 4 mg./kg. dose rate the infectivity, though reduced, was in no case completely abolished. Blood of some treated rats though revealed no trypanosomes when examined microscopically, proved infective on subinoculation. The trypanosomes examined at all subinoculations appeared to possess usual active motility.

WITH SURAMIN: In rat No. 60 the blood lost infectivity altogether at all stages of observation; otherwise the results with suramin generally followed the same trends as outlined above under observations on quinpyramine (Table II), viz., there was no loss in infectivity of blood of rats Nos. 205 and 206 treated at 4 mg./kg. body weight and rats Nos. 63 (Fig. 2) and 65 treated at 1 mg./kg. body weight. In other rats the loss in infectivity was partial.

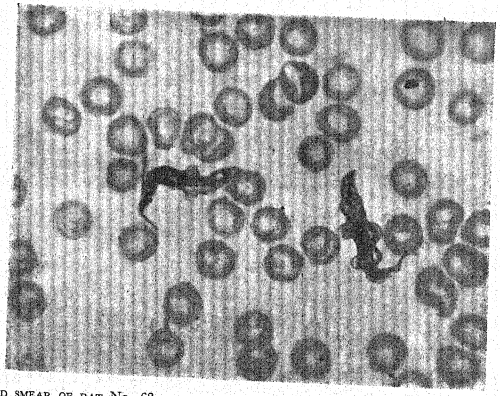

FIG. 2. BLOOD SMEAR OF RAT NO. 63 TREATED WITH SURAMIN AT 4 MG./KG. BODY WEIGHT, SHOWING BINUCLEATE AND MULTINUCLEATE FORMS OF TRYPANOSOMES WITH MORE THAN ONE FLAGELLA

TABLE II. INFECTIVITY OF PERIPHERAL BLOOD OF RATS TREATED WITH SURAMIN AT 4 MG./KG. (SERIES 1-3) AND AT 1 MG./KG. (SERIES 4)

S.	Rat No.		0 hr.	1 hr.	2hr.	4 hr.	8 hr.	12 hr.	16 hr.	24 hr.	36 hr.	48 hr.	54 hr.	60 hr.
1	405	P	T		T	T	++	+	N					
		I	6/6 (1-4)		6/6	6/6 (9-15)	6/6 (9-18)	0/6						
	407	P	T	T		T		+	N					
		I	6/6 (2-4)	6/6 (2-4)		4/6 (11-12)		3/8 (17-23)						
2	205	P		T			T		+++	+	N	N		
		I		8/8 (4-5)			7/7 (5-7)	(6-8)	7/7 (6-8)	7/7 (8-9)	8/8 (9-11)	3/5 (11)		
	206	P		T			T		+++	R	N	N		
		I		8/8 (4-5)			9/9 (4-5)		9/9 (6)	8/8 (8)	1/7 (11)	0/9		
3	58	P		+++			300			20	N	N		
		I					7/7[15] (5-9)			1/7[1] (8)	0/8	0/3		
	59	P		+++			280			10	N	N		
		I					7/7[14] (5)			7/7[1.5] (3-8)	0/2	0/6		
	60	P		+++			260			10	N	N		
		I					0/3[13]			0/8(0.5)	0/5	0/5		
4	63	P	330				520			100	60	10		N
		I					6/6[26] (4)			8/8[5] (4)	8/8[3] (4)	8/8[·5] (4)		6/6 (6)
	65	P	200				270			200	100	R		N
		I					6/6[13] (4)			6/6[10] (4)	5/5[5] (4-6)			6/6 (6)

WITH BERENIL: Partial loss in infectivity was apparent only when the flagellates were rare in blood; the prepatent period was also longer as compared to the stages of the infections nearer treatment (Table III). Some of the trypanosomes, even as early as one hour after infection, appeared devoid of motility and the undulations of the flagellum were straightened out. These specimens were presumably dead.

Mode of action of the trypanocides: The quantitative character of the infection in all the rats treated with the three trypanocides were plotted on graphs (Figs. 3-8). The infection with a trypanocide in all the rats followed consistently the same trend; therefore, graphs representing the course of infection in the two rats from each drug group are included in the paper.

It is apparent from Figs. 3-8 that the trypanosomes continued to increase in number for four to eight hours after treatment with quinpyramine and suramin and for even longer period when these drugs were used at subtherapeutic dose rates. Afterwards the counts steadily decreased and the infection was eliminated from the bloodstream within two to three days after treatment with the two drugs used at therapeutic dosages respectively. On the other hand, the trypanosomes immediately started

TABLE III. INFECTIVITY OF PERIPHERAL BLOOD OF RATS AFTER TREATMENT WITH BERENIL AT 5 MG./KG. BODY WEIGHT

S. Rat No.	0 hr.	1 hr.	2 hr.	3 hr.	4 hr.	6 hr.	8 hr.	12 hr.
1 116 P		750	540		270	150	R	N
I			4/5 (2-3)	8/8 (4-30)	8/9 (3-8)	8/8 (2-10)	6/7 (8-20)	0/7
117 P	210	160	80	30	R	N		
I	6/6	6/6	4/6	4/6	3/5	0/7		
118 P	440	430	30	20		20	N	
I			9/9 (4-18)	6/6 (4-18)	6/8 (5-15)	6/6 (8-24)	0/2	
2 400 P	1800	610	440		R	N	N	
I	6/6 (1-2)	7/7 (3-17)	8/8		3/8 (4-11)	0/7	0/7	
401 P	1216	460			N			
	6/6 (1-3)	7/9 (13-15)			0/6			
3 207 P	+++	++	R	R	N	N		
I		3/3 (4-9)	7/7 (6-9)	2/5 (9)	1/6 (9)	0/6		
208 P	+++	++	+	N	N	N		
		4/4 (6-9)	3/6 (6-9)	1/4 (6)	1/5 (6)	0/6		

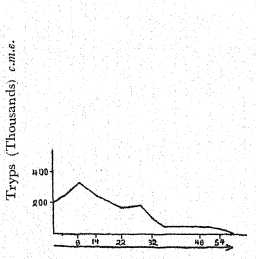

FIG. 3

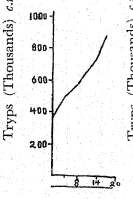

FIG. 4

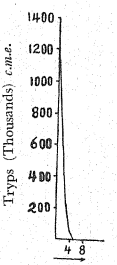

FIG. 5

FIG. 3. COURSE OF INFECTION IN RAT NO. 110 BEFORE AND AFTER TREATMENT WITH ANTRYCIDE AT 4 MG./KG. (ARROW INDICATES THE TIME OF TREATMENT—0 HR.)
FIG. 4. COURSE OF THE INFECTION IN THE CONTROL RAT NO. 500
FIG. 5. COURSE OF THE INFECTION IN RAT NO. 401 AFTER TREATMENT WITH BERENIL

decreasing in number after receiving treatment with berenil and the blood was cleared of them within eight hours of treatment. The mode of action of quinpyramine and suramin thus differed both quantitatively as well as qualitatively. Berenil acted as a cidal agent exerting its effect immediately after absorption of the lethal quantity by

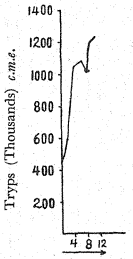

FIG. 6

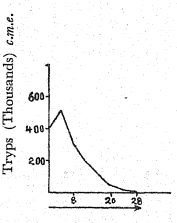

FIG. 7

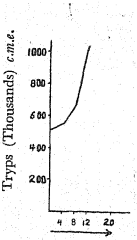

FIG. 8

FIG. 6. COURSE OF THE INFECTION OF THE CONTROL RAT NO. 215
FIG. 7. COURSE OF THE INFECTION IN RAT NO. 224 AFTER TREATMENT WITH SURAMIN AT 4 MG./KG.
FIG. 8. COURSE OF THE INFECTION IN CONTROL RAT NO. 225

the flagellates. But quinpyramine and suramin exerted their effect after a delay of four to eight hours, during which time the trypanosome counts actually increased. Their action can be easily explained by the hypothesis put forth by Hawking and Sen (1960) that suramin and quinpyramine act by blocking some biochemical system which produces an X substance essential for cell division. Unless the preformed store of the X substance is exhausted the trypanosomes continue multiplying. Hence, the action of suramin and quinpyramine after a latent period.

Changes in the morphology of treated trypanosomes: Following Ormerod (1951), trypanosomes were serially counted as normal resting forms, dividing forms (double kinetoplasts or flagella), binucleate and multinucleate forms (Tables IV and V).

TABLE IV. PERCENTAGES OF RESTING (R), DIVIDING (D), BINUCLEAR (B) AND MULTINUCLEAR (M) FORMS IN RAT NO. 401 TREATED WITH BERENIL AT 5 MG./KG.

Forms	Hours after treatment				
	0	1	2	3	4
R	85	89	91	93	95
D	8	4	2	2	5
B	3	3	2	2	2
M	..	..	..	..	..

It is apparent from Table IV that berenil treatment did not cause alteration in the morphology of the flagellates. No inclusion bodies were noted at any time in trypanosomes of any rat treated with berenil. However, both suramin and quinpyramine at some stage in post-treatment period caused delay in the cytoplasmic division with preponderance of binucleate forms. This activity, when carried further, caused

TABLE V. PERCENTAGES OF RESTING (R) DIVIDING (D), BINUCLEAR (B) AND MULTINUCLEAR (M) FORMS IN RATS NOS. 63 AND 65 TREATED WITH SURAMIN AT 4 AND 1 MG./KG. AND RAT NO. 14 AND 61 TREATED WITH QUINPYRAMINE AT 4 AND 1 MG./KG. RESPECTIVELY

Rat No.	Forms	Hours after treatment				
		0	8	24	36	48
61	R	92	95	90	90	
	B	4	5	1	2	
	D	4	0	9	8	
14	R	85	72	80	—	
	D	5	26	4	—	
	B	10	2	6	—	
65	R	86	77	94	90	
	D	4	20	4	3	
	B	10	3	2	7	
	M	—	—	—	—	
63	R	87	40	80	97	
	D	7	10	5	2	
	B	6	50	11	1	
	M	—	—	4	—	

multinucleate forms. Intensely violet staining inclusion bodies cramming the cytoplasm appeared in trypanosomes of some of the rats 4 to 48 hours after treatment with suramin and quinpyramine and then too the entire population of the trypanosomes did not develop them.

SUMMARY

The effect of berenil at 5 mg./kg., and suramin and quinpyramine both at 4 and 1 mg./kg. body weights of rats on the infectivity of *Trypanosoma evansi* to sub-inoculated mice and on the morphology of trypanosomes, and the mode of action of these trypanocides were investigated.

Berenil caused only a partial loss of infectivity and that too only when the flagellates had become rare in the blood. Quinpyramine and suramin at the lower dose rate and sometimes at the higher dose rate caused no loss of infectivity. In most of the rats treated at higher dose rates infectivity was partially lost but at no time was it completely lost as long as trypanosomes were detected microscopically. Only in one rat suramin abolished infectivity totally.

Periodical counts of trypanosomes made on rats after treatment revealed that berenil caused progressive decrease in the number of trypanosomes immediately after treatment and the blood stream was cleared of the trypanosomes within eight hours of treatment. Suramin and quinpyramine caused reduction in the number of trypanosomes after lapse of four to eight hours after treatment and the trypanosomes took two to three days to disappear from the circulation.

Berenil caused no detectable change in the morphology of trypanosomes examined after staining with Giemsa. Treatment with suramin and quinpyramine, however, delayed the cytoplasmic division following nuclear division, which caused appearance of too many binucleate forms at some stage after treatment, and even a few multinucleate flagellates could be seen.

Inclusion bodies appeared in variable proportion of trypanosomes in a few infections of rats treated with suramin or quinpyramine, but not with berenil.

ACKNOWLEDGEMENTS

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EFFECT OF ENVIRONMENTAL FACTORS ON WOOL GROWTH

III. SHEDDING OF FIBRES

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THE shedding of fibres in sheep affects their wool yield. It is also an index of the adaptability of sheep to its ambient environment. It has been reported that shedding takes place in wild and unimproved sheep. Almost all the studies on shedding in sheep have been with British breeds. Various authors have suggested that shedding or breaking of wool fibres in sheep may be due to deficient nutrition (Snell, 1936; Hardy and Earle, 1939; Allman and Hamilton, 1949; Cronje, 1959). Krishnan (1939) reported that disease or sickness may be one of the causes. Seasonal and climatic factors have also been suggested as responsible for the phenomenon (Miller, 1931; Bowstead and Larose, 1938; Lang, 1946; Burns, 1949, 1953, 1954a, 1954b, 1955; Peart and Ryder, 1954; Duncan, 1957; Wildman, 1958a, b). Genetic causes have been advanced by Ryder (1956a, b, c, 1957) and Slee (1959). Fraser and Short (1960) in their treatise on the biology of fleece reviewed in detail all the work regarding the studies on shedding of wool fibres in sheep. They summarized that in the primitive and carpet-wooled sheep there was more or less regular autumn and spring shedding of some primary fibres, probably under genetic control and sometimes reinforced by environmental stress, and a spring shedding of secondary fibres mediated by seasonal, perhaps photoperiodic conditions and also probably subject to genetic control. They stated that in some short-wool types shedding was extensive, approaching the levels found in the carpet-type fleeces, whereas in other short-wool types and in long-wool and Merino fleece types only a very small proportion of follicles was affected. Fraser and Short recommended detailed investigations to study the actual process of shedding and replacement of fibres. In the present paper the shedding in a group of Bikaneri sheep has been described.

MATERIAL AND METHODS

The observations were made on 36 adult Bikaneri ewes belonging to Chokla strain. These were divided into three groups receiving three different photoperiodic treatments of 1:2/light: darkness ratio (L_1 group), 2: 1/light:darkness ratio (L_2 group) and Control group. The details regarding the treatments and management have already been described earlier (Nagarcenkar and Bhattacharya, 1964a, b). The

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observations on shedding were maintained periodically as the symptoms were manifested. Photographs were also taken to depict the process. Skin samples were obtained from three ewes belonging to L_2 group. These were processed and horizontal sections were obtained as per the method of Carter and Clarke (1957) with slight modifications for the duration of dehydration, clearing and impregnation.

RESULTS

Incidence of fibre shedding was noticed first in the L_2 group of ewes receiving light for 16 hours continuously. It was observed earliest at the end of July in ewe No. 112. Later, in early August, it was seen that some more ewes also revealed shedding of fibres. Observations were then made from time to time and these have been presented in Table I. Photographs of animals in different stages of shedding are presented in Figs. 1-4. Skin sections from the three ewes of the L_2 group were obtained with a biopsy punch on August 21, 1959, from the sites described below.

Ewe 88: Skin section was obtained from right side breech region. The fibres in this region were shed already.

Ewe 112: Skin section was obtained from left side body region, at the junction of the last rib with the costal cartilage. Fibres from this site were not yet shed, although much shedding had already taken place on other regions and the appearance of the animal was as if almost naked.

Ewe 119: Skin section was obtained from the right side of the shoulder region. Fibres from this site were already shed.

Fig. 5 shows that the shedding of fibres was due to intrinsic behaviour of the follicles. Bundles of empty or partially empty follicles were observed in case of all the three ewes. The sequence of shedding of fibres may be described as follows: In the beginning there is rise of fibres along the mid-dorsal line, followed by rise along the flanks and other body regions. The incidence of shedding occurs first on the neck and then on the shoulder. In some cases it may take place earlier on the shoulder. Later, the shedding appears on the breech region and simultaneously on middle of the tail or at root of the tail in a few cases. After this the shedding on the upper body region starts and proceeds ventrally. The shedding process is bilateral and proceeds in the same fashion on either side of the animal's body. It seems that if left to itself the animal would completely slough off its fleece provided it is kept in congenial climatic conditions.

DISCUSSION

The results presented here have shown an intense shedding of wool fibres in the L_2 group of ewes. Some shedding was also observed in the L_1 group but no evident shedding was observed in the control group. It may be stated here that in the previous year the fleece from the same flock of sheep was not shorn for over one year. However, no shedding was perceptible, though there was strong *cotting* of the fibres, which means that some of the fibres were shed and were held together by the other adjoining fibres. The fleece did not slough off due to the delayed shearing, as was observed in the present investigation. This conforms with the view of other workers regarding

TABLE I. OBSERVATIONS ON SHEDDING OF WOOL FIBRES

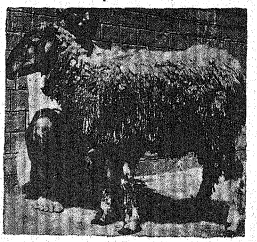
Date	Groups			Remarks
	L_2 Group	L_1 Group	Control Group	
27–7–1959	Ewe 112 Showed first incidence of shedding. Observed first on left side of neck and partly on right side of neck and shoulder			
5–8–1959	Ewe 88 Shedding on both sides on shoulder patch.			
	Ewe 112 Shedding in much advanced stage, observed on both sides on neck and shoulder. Shedding on breech region and head of tail also observed.			
	Ewe 119 On the neck region on both sides and on right shoulder.			
	Ewe 217 On the neck region.			
19–8–1958	Ewe 88 Shedding on neck and shoulder regions on both sides. On the body and breech regions just developing.		Rise of fibres seen in almost all the animals on the dorsal line along the vertebral column, in varying degrees.	No shedding observed. No rise of fibres also.	Ewes 117, 170, 215 and 248 from the Control group shorn on 20-8-59.
	Ewe 112 Complete shedding on neck, shoulder and on upper body region. Shedding on breech region in progressed stage.				Ewes 187 and 235 from L_2 group and ewes 78, 98 and 185 from the L_1 group shorn on 21-8-59.
	Ewe 119 Shedding on neck, shoulder and breech regions on both sides. Rise of fibres along the dorsal line.				Ewes 112 and 119 from L_2 group and ewe 128 from L_1 group shorn on 22-8-59.
	Ewe 188 Shedding just starting on the shoulder region on either side.				Skin samples from ewes 88, 112 and 119 from L_2 group collected on 21-8-59.
	Ewe 190 Shedding just starting on the shoulder region on the left side only.				
	Ewe 217 Shedding on the neck and shoulder regions extending posteriorly and just developing on the breech region				
	Ewe 235 Shedding on neck and shoulder on left side and only on shoulder on right side.				
	Ewes 187 and 233 No shedding. Rise of fibres along the mid-dorsal line and on the flanks.				
	Ewes 214 and 260 No shedding. Slight rise of fibres along the mid-dorsal line.				
	Ewe 232 No shedding. No rise of fibres.				
20– 9–1959	Ewe 88 Shedding on the neck, shoulder, upper body region, and on breech.	Ewe 201 Shedding on head of tail and breech. Shedding just starting on the neck region.		No shedding observed and no perceptible rise of fibres seen.	Ewes 88, 190, 214 and 260 from L_2 group and ewes 45, 94, 166 and 205 from L_1 group shorn on 21–9–59.

TABLE I (*Concld.*)

Date	Groups								Remarks
	L_2 Group			L_1 Group			Control Group		
	Ewe	188	Shedding on the shoulder and on the tail. Slight incidence on breech region.	Ewe	203	Shedding on neck on both sides. (First incidence observed on 10–9–59).			Ewes 110, 152, 200 and 249 from Control group shorn on 20–9–59.
	Ewe	217	Shedding on the neck, shoulder and breech regions. Rise of fibres in all the rest unshorn ewes.	Ewe	205	Shedding on body region, neck and breech. (First symptom observed on 24–8–59). Rise of fibres in all the rest unshorn ewes.			
28–9–1959	Ewe	188	Complete shedding of fibres on the tail, shedding on the shoulder.	Ewe	121	Complete shedding of fibres on the tail; shedding on the neck and shoulder just starting.			
18–10–1959	Ewe	188	Shedding on shoulder slightly further advanced. Shedding on breech also started. Complete shedding on the middle of the tail.	Ewe	121	Complete shedding on tail; shedding on neck and shoulder not much progressed. Rise of fibres along the mid-dorsal line conspicuous.	No shedding still in the unshorn animals.		Rest of the animals shorn on 18th, 20th and 21st October, 1959.
	Ewe	217	Very pronounced shedding on the neck. Shedding on shoulder, breech and head of tail.	Ewe	201	Shedding on neck very prominent. Shedding also on shoulder and breech regions.			
	Ewes and	232 233	Rise of fibres along the mid-dorsal line.	Ewe	203	Shedding on neck, on the middle of tail just starting.			
				Ewe	273	No shedding. Rise of fibres on the mid-dorsal line.			

1

2



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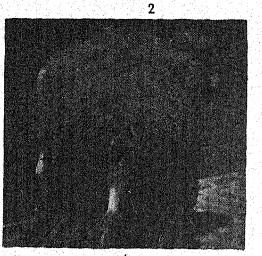

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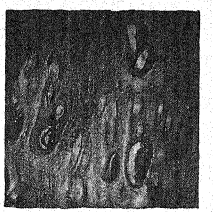

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FIG. 1. RISE OF FIBRES ALONG MID-DORSAL LINE
FIG. 2. SHEDDING ON THE NECK AND SHOULDER REGION
FIG. 3. EXTENSIVE SHEDDING ON THE NECK, SHOULDER AND BREECH REGIONS AND PARTLY ON THE BODY REGION
FIG. 4. SHEDDING ON MIDDLE OF THE TAIL AND RISE OF FIBRES ALONG THE MID-DORSAL LINE
FIG. 5. MICRO-PHOTOGRAPH OF SKIN SECTION DEPICTING SOME EMPTY AND PARTIALLY EMPTY FOLLICLES (×60)

the carpet-wooled sheep. Iljin (1938) even proved that the threshold stimulus required for inducing shedding varied according to the fibre types, fine wool fibres requiring lesser stimulus as compared to the strong kempy fibres. From his investigation on Masham sheep (a carpet-wooled breed), Ryder (1956a) reported that the

secondaries were shedding throughout the year. In the skin sections obtained by the authors in certain cases there were whole bundles of empty secondary follicles. The skin section presented in Fig. 5 discloses follicles in different stages of shedding, indicating that the process of shedding was not sudden but sequential. The data presented in Table I show inter-group as well as intra-group differences in animals in the incidence and intensity of shedding. The shedding in the animals was not due to any nutritional deficiency, as they were given their full nutritive requirements and were in good health.

Narayan (1960) observed shedding of follicles in skin specimens obtained in autumn from Chokla, Malpura and Marwari type Bikaneri sheep from Rajasthan. Krishna Rao *et al.* (1960) did not find shedding in skin specimens obtained in spring from Bikaneri sheep from Madras and Andhra Pradesh. Data obtained in the present investigation also reveals that the shedding and later sloughing off of the fleece was observed in autumn, when there was high environmental temperature in conjunction with high humidity. It may be conjectured that the shedding may be a result of the interaction of the photoperiod with the climatic conditions, a larger photoperiod as in L_2 group stimulating earlier and intense shedding. From this view-point it may be stated that there was no shedding in animals in Control group, as they were receiving a gradually declining natural photoperiod in the autumn season. This compensative mechanism, therefore, did not induce any shedding in the animals belonging to this group. The data of Slee and Carter (1961) on Wiltshire Horn sheep is also in agreement with the present findings in that they found maximum shedding in May-July, which coincided with longer day lengths and higher atmospheric temperatures. The other possibility could be that, as stated earlier (Nagarcenkar and Bhattacharya, 1964b), any change in photoperiod, as in L_1 and L_2 groups, over-stimulates the pituitary gland, and due to regeneration of fibres the old fibres are pushed off and shedding results. The observations of Wright (1954), wherein he found shedding in Merino fleece a non-breaking fleece type under high environmental temperatures in Iraq, are in agreement with the above propounded theory. Hammond Jr. (1952) reported that in ferrets high environmental temperature contributed more to shedding as compared to the photoperiod. Yeates (1955) observed shedding of coat in Poll Shorthorn calves 10–12 weeks after the days began to lengthen. Slee (1959) also found that shedding of fibres in Wiltshire Horn sheep occurred between June 8 and 30, i.e., close to the longest day. Slee believed shedding to be controlled by polygenic genes. He, however, stated that the environmental factors were also important. Most of the literature on shedding reported the occurrence of the phenomenon in autumn. It has been reported earlier (Nagarcenkar, 1963) that high temperature, humidity and photoperiod depress thyroidal secretions and possibly increase adrenal secretions, which in turn affect the wool growth. Maqsood (1950) observed that in hypothyroid Suffolk sheep the wool was easily pluckable. Lindner and Ferguson (1956) proved by injecting experimental sheep with adenocorticotropic hormone, which induced large-scale shedding of fibres, that the seasonal break in fleece, rising of the fleece and ' cotted ' fleece were due to increased activity of adrenal cortex. Underwood (1960) also stated that break in wool took place due to temporary overactivity of the adrenal cortex in response to the environmental stress. Individual variations

in shedding intensity in sheep as reported herein have also been observed by Ryder (1956a), Burns (1954b, 1955), and Slee (1959).

SUMMARY

Observations on shedding of fibres in 36 Bikaneri ewes were maintained and their sequence has been described. An intense incidence of shedding with sloughing off of fleece was observed in the L_2 group ewes, to a lesser degree it was seen in the L_1 group ewes and no perceptible shedding was found in the Control group ewes. Shedding was observed only in the autumn season, when high temperature and high humidity prevailed. Variations in the animals of both the L_2 and L_1 groups of ewes regarding the shedding intensity were also observed.

It is conjectured that the shedding is a result of the interaction of photoperiod × temperature × humidity. An increase in these factors depresses the thyroid activity and increases the adrenal gland secretions, which inhibit the wool growth, and the fibres thus detached from the follicles are sloughed off. Another reason advanced is that any change in the photoperiod over-stimulates the pituitary, which induces regeneration of fibres, pushing out the old ones which, in turn, results in shedding.

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STUDIES ON THE EFFICACY OF DIFFERENT ANTHELMINTICS AGAINST *MONIEZIA EXPANSA* IN SHEEP

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THE comparative efficacy of different anthelmintics commonly used against cestode infections was studied with a view to finding out the cheapest, non-toxic and most efficacious drug against *Moniezia expansa* in sheep.

MATERIAL AND METHODS

Lambs and sheep naturally infected with the parasite were maintained under parasite-free conditions. The animals were muzzled throughout the experiment except when they were given feed and water. They were given exercise during the day. Green leaves, mostly of *pakhar* or berseem, were given to them *ad lib.*

Each treatment group consisted of four animals, of which one was kept as infected control without treatment. In some treatment groups, however, one or two experimental animals were added either to confirm the results already achieved or to try the same drug at a different dose level. Animals in each group had more or less equal body weights, which avoided variance in the quantity of dose administered. The intensity of infection in different groups was observed for about a month before administration of the drug by counting the segments of the parasites discharged in the faeces of individual animals. The observations on the segments discharged were continued from one to two weeks after the treatment continuously till their death or slaughter.

The drugs tried were classified on the basis of their chemical composition as under:

(a) *Anthelmintics of metallic origin:* Copper arsenate, copper sulphate, Paris green, arsenic trisulphide (yellow), lead arsenate, tin arsenate, and tin arsenite.
(b) *Anthelmintics of non-metallic origin:* Sodium arsenate and calcium arsenate.
(c) *Anthelmintics of plant origin:* Liquor filicis and nicotine sulphate (in combination with copper sulphate).

The percentage efficacy of the drugs was calculated on the basis of the number of immotile parasites passed following the treatment and the motile number remaining unaffected in the intestines of the animals as recovered at autopsy. Segments or incomplete worms were not taken into account.

The animals were fasted for 18 hours prior to the administration of the anthelmintics. The drugs not possessing purgative action were followed by purgatives, such as liquid paraffin or castor oil. The drugs were administered orally. Poisonous drugs were given in gelatin capsules. During the trial the body weight, temperature and general condition of the animals were recorded.

RESULTS

The data showing comparative efficacy of different drugs are presented in Table I.

TABLE I. COMPARATIVE EFFICACY OF DIFFERENT DRUGS AGAINST *MONIEZIA EXPANSA* IN SHEEP

Anthelmintics	No. of naturally infected sheep and lambs treated	Dose per animal	No. of worms recovered after treatment	No. of worms present at autopsy	Total No. of worms present	Efficacy of the drug (per cent)	Route of administration	Cost of the drug per lb. (in Rs.)
Mixture of copper sulphate and nicotine* sulphate in equal parts	4	45–60 cc.	6	14	20	30	Drench	*39·00
Copper arsenate	3	0·5 gm.	3	2	5	60	Capsule	25·00
Paris green	4	0·15–0·3 gm.	4	1	5	80	Capsule	20·00
Arsenic trisulphide	2	0·5 gm.	3	1	4	75	Capsule	19·50
Copper sulphate	2	75 cc.	..	5	5	0	Drench	1·00
		(The drug was found to be poisonous)						
Liquor filicis 25% W/w in castor oil	2	0·5 gm.	..	..	14	0	Drench	16·00
Sodium arsenate	4	0·5–1·0 gm.	1	13	14	7·14	Capsule	14·00
		(The drug was found to be poisonous)						
Calcium arsenate	3	0·3 gm.	4	Nil	4	100	Capsule	19·00
	3	0·15–0·2 gm.	4	Nil	4	100	Capsule	
Lead arsenate	3	0·5–1·0 gm.	5	Nil	5	100	Capsule	37·00
	4	0·5 gm.	9	Nil	9	100	Capsule	
Tin arsenate	2	0·5 gm.	6	Nil	6	100	Capsule	48·00
Tin arsenite	3	0·3 gm.	9	Nil	9	100	Capsule	52·00

DISCUSSION

Anthelmintics of plant origin: Two drugs, namely, liquor filicis in castor oil and nicotine sulphate in combination with copper sulphate were tried against the parasite. Liquor filicis 25 per cent W/w in castor oil was not at all efficacious against *M. expansa*. However, it accelerated the rate of discharge of segments of the parasite.

Nicotine sulphate in combination with copper sulphate (equal parts of 40 per cent nicotine sulphate and 2 per cent copper sulphate solution) given at the rate of 45 to 60 cc. per sheep showed only 30 per cent efficacy against *M. expansa*. The drug was tried by Daubney (1923, 1929) and Freeborn and Berry (1934), but they did not mention its percentage efficacy against the parasite. Sen (1943) used 1 per cent copper sulphate solution mixed with 40 per cent nicotine sulphate at the dose of 85 gm. per sheep, but did not mention the efficacy of the drug. Thomas *et al.* (1956) found that commercial preparation of copper sulphate and nicotine sulphate given at the rate of 28 gm. per lamb was reasonably effective. Fair results on large scale were obtained with a mixture of equal parts of 1·5 per cent copper sulphate and 40 per cent nicotine sulphate (Lapage, 1956).

Anthelmintics of non-metallic origin: Two drugs, namely, sodium arsenate and calcium arsenate, were tried against *M. expansa*. Sodium arsenate given at the dose of 0·5 gm. per sheep was found to be toxic to the host. None of the animals thus treated survived. The drug was found to be only 7·14 per cent effective against the parasite. Daubney (1923) advocated sodium arsenate with copper sulphate in the ratio of 1:4 at the rate of three grains per lamb, repeated next day, but did not mention its efficacy against the parasite or its toxicity to the host.

Calcium arsenate has been found to be 100 per cent efficacious against *M. expansa* in sheep as well as in lambs when given at the rate of 0·3 gm. per sheep and 0·15 - 0.2 gm. per lamb. The drug was not toxic to the host. It was interesting to note that all the worms of this species were eliminated along with their scolices within 48 hours after the treatment. However, the drug did not have any effect on the worms of the genus *Stilesia*. The results confirm the findings of Akramovski *et al.* (1957) and Egorov and Bobkova (1959), who also observed 100 per cent efficacy of the drug against this parasite in sheep and lambs, at the dose rate of 0·3 –0·5 gm. per animal.

Anthelmintics of metallic origin: The following drugs were tried against *M. expansa* in sheep: copper sulphate, Paris green, copper arsenate, lead arsenate, arsenic trisulphide, tin arsenate and tin arsenite.

In the present investigations 2 per cent copper sulphate given at the dose rate of 75 cc. per sheep weighing 30–35 lb. was found to be poisonous. However, Skriabine and Schulz (1934) found it 98 per cent effective when given at a lower percentage in two doses at the interval of ten days following saline purgative. Gordon (1935) reported 66·7 per cent efficacy of this drug. Ozerskaya (1944), Ikkol (1946), Istomin (1946), Potemkina (1946; 1951), Katich (1947), Lafenetre (1948), Paskalskaya (1959) and Svadzyhyan *et al.* (1960) obtained good results by giving 1 per cent solution of this drug at the varying dose rates against this parasite.

Paris green was found to be 80 per cent effective against *M. expansa* but proved fatal to sheep even at a lower dose of 0·15 gm. per animal. Of the four experimental

animals treated, three died due to its toxicity. The results are contrary to the findings of Akramovski *et al.* (1957), Kesler (1959), and Shcherbatyuk and Kabaev (1959), who reported 100 per cent efficacy of this drug when given at dose of 0·1–0·7 gm. to lambs and sheep.

Copper arsenate has been found to be 60 per cent efficacious against mature *M. expansa* in sheep at a dose of 0·5 gm. per sheep. It did not show any anthelmintic activity against immature worms of this species. Akramovski *et al.* (1957), however, reported complete cure in lambs when treated with this drug at a dose rate of 0·3–0·5 gm. per lamb.

Lead arsenate was found 100 per cent efficacious against *M. expansa* at the minimum dose level of 0·5 gm. per sheep and lamb. It was also found to be harmless and efficacious to the same degree at a dose of 1 gm. per animal. These investigations conform to the findings of McCulloch and McCoy (1941), Radeleff (1944), Ward and Scales (1946), Allen and Jongeling (1948), Link *et al.* (1950), Morgan *et al.* (1950) and Maghami *et al.* (1959).

Arsenic trisulphide has been found to be 75 per cent efficacious against this parasite when given at a dose of 0·5 gm. per sheep. Gordon (1935), however, reported 100 per cent efficacy of this drug when given at 0·5 gm. per sheep either as a powder or suspension in 2 per cent copper sulphate solution.

Tin arsenate and tin arsenite have been found to be 100 per cent effective against *M. expansa* in sheep and lambs when given at the dose rates of 0·5 and 0·3 gm., respectively. The drugs were found to be harmless to the animals. The results conform to the findings of Chubabriya (1955), Akramovski *et al.* (1957) and Chubabriya and Goderdzishivili (1959) in respect of tin arsenate when given at the rate of 0·3–0.8 gm. per sheep. Svadzhyan *et al.* (1960) found that tin arsenate was 65 to 79 per cent efficacious against *M. expansa* in lambs. Garkavi (1956) reported complete cure against this parasite in sheep, when tin arsenite was given at the dose of 0·4–0·6 gm. per kg. body weight in gelatin capsule per sheep. He also stated that even 2 gm. of this drug was harmless to lambs. The findings of Garkavi (1956) conform to the findings in the present investigations in respect of tin arsenite.

CONCLUSIONS

Of the 11 drugs tried calcium arsenate, lead arsenate, tin arsenate and tin arsenite were 100 per cent efficacious against *M. expansa* in sheep and lambs. Besides, they were harmless to the host in the doses recommended. Out of these, calcium arsenate was the cheapest, its cost being Rs. 19 per lb., while the cost of the other three drugs ranged from 39 to 52 Rs. per lb. Calcium arsenate is, therefore, recommended for general treatment of lambs and sheep infected with *M. expansa* in doses given in this paper.

SUMMARY

A mixture of equal parts of 2 per cent copper sulphate and 40 per cent nicotine sulphate at the dose rate of 45–60 cc. was 30 per cent efficacious against *M. expansa*.

Copper arsenate at a dose rate of 0·5 gm. per sheep weighing 20–30 lb. had 60 per cent efficacy against the parasite. It did not have anthelmintic action on the immature worms of *M. expansa.*

Sodium arsenate (0·5–1 gm.) was found to be poisonous to the host and had very little efficacy against *M. expansa.*

Copper sulphate (2 per cent) in a dose of 75 cc. per sheep weighing 30–35 lb. was found to be poisonous to the host. It had no anthelmintic action against *M. expansa.*

Liquor filicis 25 per cent W/w given at the rate of 0·5 gm. in 1 oz. of castor oil per sheep did not have any action on *M. expansa.* The treatment, however, accelerated the rate of discharge of segments.

Paris green 0·15 to 0·3 gm. per sheep had 80 per cent efficacy against the parasite, but there was 66·6 per cent mortality in the treated animals within four days of treatment.

Calcium arsenate at a dose of 0·15–0·2 gm. per lamb and 0·3 gm. per sheep given in gelatin capsule was 100 per cent efficacious against *M. expansa.* The drug did not have any anthelmintic action on worms of the genus *Stilesia.* It was harmless to the host. Considering the comparative cost of other 100 per cent efficacious anthelmintics against the parasite, this anthelmintic was cheapest.

Lead arsenate, 0·5–1·0 gm., per sheep was harmless to the host and 100 per cent efficacious against *M. expansa.* The parasites were removed within 24 hours after treatment.

Arsenic trisulphide (yellow) in a dose of 0·5 gm. per sheep was 75 per cent efficacious against *M. expansa.* The drug was harmless to the host. It did not have any anthelmintic action against immature worms.

Tin arsenate at the dose rate of 0·5 gm. per sheep was 100 per cent efficacious against *M. expansa.* The drug was non-toxic to sheep.

Tin arsenite at the dose rate of 0·3 gm. per lamb was 100 per cent efficacious against *M. expansa.* This drug was also non-toxic to lambs.

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FACTORS AFFECTING MILK PRODUCTION IN SAHIWAL COWS

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Milk production in a dairy cow is of basic economic importance. Though an inherited character, it is highly influenced by environmental factors at various stages. Several workers in Egypt have studied the effect of various non-genetic factors that influence the milk yield of buffaloes (Ragab *et al.*, 1953, 1954a; Alim and Ahmed, 1954; Alim, 1957; Asker and El-Itriby, 1958), but very little work has been done on this subject on our Indian cattle, especially on the Sahiwal cows.

This investigation was, therefore, undertaken in 1960–61 to study the effect of the following factors on the milk production of Sahiwal cows:

(i) age at first calving, (ii) lactation period, (iii) dry period, (iv) peak yield, and (v) month of calving.

MATERIAL AND METHODS

The data on Sahiwal herds of cattle were collected from Military farms at Lucknow, Meerut, Ambala and the Chakganjaria Farm, Lucknow. The management and feeding on these farms was done according to the standards laid down by the experts. Statistical methods of analysis of variance, correlation and regression were used for studying the effect of the above factors on milk production (Snedecor, 1956). The genetic correlations have been completed by the method developed by Hazel (1943) and described by Hazel *et al.* (1943). The heritability estimates have been calculated by half-sibs correlation method outlined by Hazel and Tessill (1946).

RESULTS AND DISCUSSION

Effect of age at first calving on milk production in the first lactation: The number of heifers available for this study was 160 and the average age at first calving was $38 \cdot 45 \pm 0 \cdot 39$ months. This estimate is higher than that reported by Ragab *et al.* (1954b), but is definitely lower than the estimate of 47·3 months found by Venkayya and Anantakrishnan (1956) for the Gir breed in India. Joshi and Phillips (1953), however, have shown that in most Zebu breeds of cattle the heifers usually calve when they are over 40 months of age.

The heifers according to their age at first calving were classified (Fig. 1) into 12 groups with a class interval of two months. The youngest group included animals calving for the first time up to 30 months and the last one above 50 months. The percentage of heifers in each group along with their average age at first calving and the corresponding milk yield during first lactation has been given in Table I. The distribution of age at first calving showed that about 52·4 per cent of the heifers studied calved below 38 months of age whereas nearly 47·6 per cent calved above it.

TABLE I. EFFECT OF AGE AT FIRST CALVING, LACTATION PERIOD, DRY PERIOD AND PEAK YIELD ON MILK PRODUCTION

Group	Age at first calving (md.)			Lactation period (days)			Dry period (days)			Peak yield (lb.)		
	Per cent heifers	Group age in months	Av. M. Y. (lb.)	Per cent heifers	Group L. P. in days	Av. M. Y. (lb.)	Per cent heifers	Group D. P. in days	Av. M. Y. (lb.)	Per cent heifers	Group P. Y. in lb.	Av. M.Y. (lb.)
1	1·2	Up to 30	3,354	4·9	Up to 210	2,066	6·4	Up to 75	3,841	8·0	Up to 12	2,661
2	3·1	31–32	4,120	3·7	211–225	2,602	26·6	76–90	4,518	13·6	13–14	2,610
3	14·2	33–34	3,262	7·5	226–240	2,907	14·2	91–105	4,603	19·7	15–16	3,312
4	16·0	35–36	3,723	9·3	241–255	3,338	15·2	106–120	4,225	16·2	17–18	3,544
5	17·9	37–38	3,571	14·8	256–270	3,430	9·2	121–135	4,815	12·9	19–20	3,858
6	13·6	39–40	3,655	12·9	271–285	3,602	8·0	136–150	4,237	9·9	21–22	4,326
7	15·4	41–42	3,910	22·8	286–300	3,874	7·5	151–165	4,633	8·6	23–24	4,671
8	7·4	43–44	3,606	8·0	301–315	4,317	2.6	166–180	4,369	4·9	25–26	4,833
9	3·1	45–46	3,858	9·3	316–330	4,461	3·3	181–195	4,087	3·7	27–28	4,814
10	4·3	47–48	3,925	6·8	above 330	4,954	1·4	196–210	5,095	2·5	above 28	5,653
11	1·8	49–50	3,894	..	..	..	..	above 210	4,929	..	..	..
12	2·0	above 50	4,177	..	..	..	..	..	..	..	..	..

Asker *et al.* (1958), while analysing data for age at first calving of native cows, found that about 42 per cent of the heifers calved below the model group while 36 per cent calved about it. For Gir breed Venkayya and Anantakrishnan (1958) found it to be 6·5 and 71·0 per cent below and above the model group respectively.

The analysis of variance given in Table II clearly indicates that there were significant differences between the groups as far as the effect of age at first calving on milk yield during first lactation is concerned. The genetic correlation between these two traits was also found to be significantly high (0·8126).

TABLE II. ANALYSIS OF VARIANCE FOR THE EFFECT OF AGE AT FIRST CALVING OF MILK PRODUCTION

Source of variance	Degrees of freedom	Sum of squares	Mean square	F
Total	161	182,735,252	..	..
Before farms	2	1,314,485	..	..
Within farms	159	181,420,767	..	..
Between groups	28	65,294,117	2,331,932·75	2·631**
Within groups	131	116,126,650	886,462·98	..

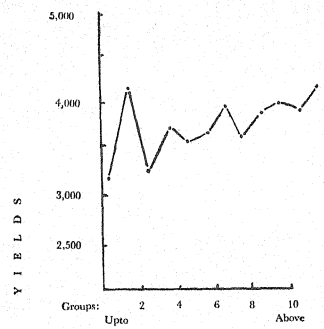
**=Significant at P/ 0·01

Results obtained in this study are in close agreement with the findings of workers in study on Zebu and dairy cattle in temperate and tropical zones (Champan and Dickerson, 1936; Mahadevan, 1951, 1953, 1956; Venkayya and Anantakrishnan, 1956) while, in contrast, Ragab *et al.* (1954b) and Asker *et al.* (1958) observed non-significant correlation between these traits.

The heritability of age at first calving was found to be 0·5043±0·1938, which is quite high. The high heritability strongly suggests that the genetic improvement in these animals for this trait can be brought about very quickly by appropriate individual selection. Any reduction in the age at first calving not only minimizes the capital investment in terms of the cost of raising the heifers to their productive life, but also shortens the generation interval which is desirable for progeny-testing of bulls. This is one of the main problems of Indian cattle population.

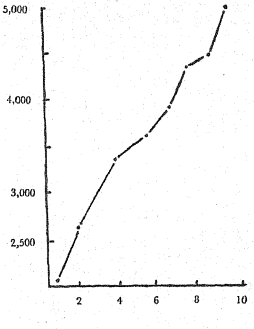
Effect of lactation period: The average lactation period in this study was found to be 295·8±4·7 days with a coefficient of variation of 23·8 per cent. For finding out the effect of lactation period on milk yield, the data of lactation period was classified into ten groups, with a class interval of 15 days. The first group includes lactations which are less than 210 days, while the last one includes those of 330 days and above.

It is evident from Table I and Fig. 2 that the milk yield increases along with the increase in the lactation period. The genetic correlation between these two traits was found to be 0·6382, which is highly significant. This is further supported by the highly significant (P/0·01) differences between the groups, as shown by the analysis of variance given in Table III.



<30 (in 2-month interval) 50>

FIG. 1. EFFECT OF AGE AT FIRST CALVING ON MILK YIELD.



<210 (in 15-day interval) 330>

FIG. 2. EFFECT OF LACTATION PERIOD ON MILK YIELD.

TABLE III. ANALYSIS OF VARIANCE FOR THE EFFECT OF LACTATION PERIOD OF MILK YIELD

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Total	161	182,735,119	..	..
Between farms	2	26,601		n.s.
Within farms	159	290,379		
Between groups	26	92,411,309	3,554,281	5·3149**
Within groups	133	88,942,123	668,738	..

**Significant at P/ 0·01

Similar observations have been reported by Sikka (1931), Robertson (1950), Mahadevan (1956) and Asker *et al.* (1958). Such high genetic correlation indicates that the selection on milk yield would also simultaneously bring about good genetic gain in the length of lactation period, which is also an indication of persistancy in milk production.

Effect of preceding dry period: The average dry period for Sahiwal cattle in this study was found to be 121·2±3·8 days and the coefficient of variation came to 47·3 per cent. The high variability in this character was reported by several workers in their studies on dairy cattle, Zebu cattle and buffaloes (Mahadevan, 1953, 1956; Ragab *et al.*, 1954; El Itriby and Asker, 1958).

For studying the effects of dry period on the milk yield in the subsequent lactation, the data of the dry period was classified into 11 groups with a class interval of 15 days. The both extreme classes were those that included period up to 75 days and above 210

days. The relation between the preceding dry period and milk yield has been graphically presented in Fig. 3, which indicates that the milk yield was maximum following a dry period of 90–105 days and thereafter the trend was not constant but on averaging was found to be gradually declining. The differences between the groups of milk yield according to the analysis of variance given in Table IV were non-significant.

TABLE IV. ANALYSIS OF VARIANCE FOR THE EFFECT OF PRECEDING DRY PERIOD ON MILK YIELD

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Total	161	31,066,166	..	..
Between farms	2			
Within farms	159			
Between groups	26	5,246,931	201,805	1·053n.s.
Within groups	133	25,485,019	191,617	..

n. s. = Non-significant.

Effect of peak yield: The average peak yield in this study was found to be 18·3 ± 0·37 lb. with a coefficient of variation of 25·68 per cent. The variation in the milk yield due to a peak yield was investigated by tabulating peak yield into ten classes with a class interval of 2 lb. The first group included peak yield up to 12 lb. while the last group comprised of more than 28 lb.

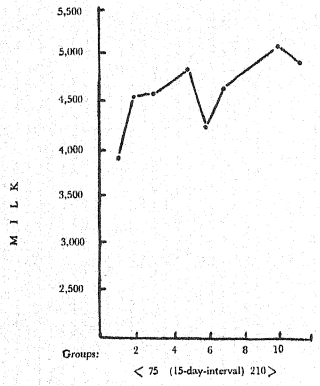


FIG. 3. EFFECT OF PRECEDING DRY PERIOD ON MILK YIELD.

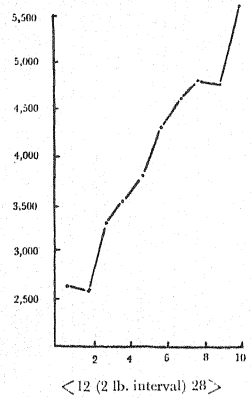


FIG. 4. EFFECT OF PEAK YIELD ON MILK YIELD.

The genetic correlation of 0·6072 between peak yield and milk yield was found to be highly significant. Table I and Fig. 4 indicate that increase in the peak yield was closely associated with increase in the milk yield of cows. This is further supported by the highly significant differences between the groups of peak yield, as shown by analysis of variance presented in Table V.

TABLE V. ANALYSIS OF VARIANCE FOR THE EFFECT OF PEAK YIELD ON MILK YILED

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Total	161	182,735,119	..	..
Between farms	2	197	..	n.s.
Within farms	159	3,394	..	..
Between groups	25	112,454,380	4,498,175	8·748**
Within groups	134	68,899,052	514,172	..

** Significant at P/ 0·01

This is in agreement with the observations of Sikka (1950) and Ullah (1952). Such a high relationship is of great significance because not only preliminary selection of first calvers can be done on their peak yield and culled animals can be disposed off easily so long as they are in milk, but the breeding bulls also can be progeny tested much earlier before the daughters complete their lactations.

Effect of month of calving: The study of the effect of month of calving on milk production of cows calved at different months of the year, along with the average milk production and their relative yield, compared to the mean of all animals was made. The data is given in Table VI.

TABLE VI. EFFECT OF MONTH OF CALVING ON MILK PRODUCTION

Month	No. of lactations	Average milk yield (lb.)	Relative yield
January	67	4,573	110·5
February	55	4,457	107·7
March	73	3,962	95·8
April	48	3,919	94·8
May	29	4,007	96·9
June	31	4,083	98·7
July	34	4,060	98·3
August	27	4,226	102·2
September	43	3,916	94·7
October	28	4,364	105·5
November	45	3,655	88·4
December	45	4,403	106·5
Total and average	525	4,135	100·0

It is quite evident from Table VI that the cows calving during the months of December, January and February, i.e., winter months produced higher milk yield than the average, while those calving during the months of March, April and May produced about 4 per cent less milk than the average. Table VII showing the analysis of variance for the effect of month of calving on milk indicates that the differences between the groups were found to be non-significant.

TABLE VII. ANALYSIS OF VARIANCE FOR THE EFFECT OF MONTH OF CALVING ON MILK YIELD

Source of variance	Degrees of freedom	Sum of squares	Mean square	F
Total	524	878,361,897	..	..
Between months	11	27,853,692	2,532,154	1·527n.s.
Within months	513	850,508,205	1,657,911	..

n. s. = Non-significant.

Ragab *et al.* (1954a), Alim and Ahmed (1954) and Asker and El-Itriby (1958) observed non-significant effect of month of calving on milk yield in buffaloes. The results of the present study are in agreement with the above-mentioned authors and also with Asker *et al.* (1958), but not in agreement with the findings of Ragab *et al.* (1954b).

Month of calving will only have significant effect on milk yield when there are extreme variations in weather conditions and the availability of food at different months of the year. These effects on milk yield are diluted by analyzing them month-wise basis. However, when these are grouped together according to season, these differences are widened to the extent of little more than 10 per cent, which is quite significant.

SUMMARY

The study of the records of 160 cows of Sahiwal breed was made to find out the influence of various characters on milk production. The results of this investigation indicate that the average age at first calving in these herds is $38 \cdot 45 \pm 0 \cdot 39$ months and it is highly correlated genetically with first lactation yield to the extent of $r = 0 \cdot 8126$. This strongly suggests that simultaneous selection for both the traits is most essential. The average peak yield and lactation period were $18 \cdot 3 \pm 0 \cdot 37$ lb. and $295 \cdot 8 \pm 4 \cdot 7$ days respectively. The genetic correlations of these traits with milk yield were $0 \cdot 6072$ and $0 \cdot 6382$ in the same order. These findings indicate that peak yield can be taken as preliminary measure of selecton for milk yield. The average dry period was found to be $121 \cdot 2 \pm 3 \cdot 8$ days. It was also observed that maximum yield followed an optimum dry period of 90 to 105 days. Either shorter or longer period than this showed a declining trend in yield.

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STUDIES ON POST-PARTUM OESTRUS IN MURRAH BUFFALOES

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THE interval from parturition to first post-partum oestrus is quite variable in bovine as reported by several workers (Chapman and Casida, 1935; Trimberger, 1941; Herman and Edmondson, 1950; Old and Seath, 1953; Buch *et al.*, 1955). However, knowledge concerning the post-partum oestrus in buffaloes is scanty. Hafez (1952) reviewed the findings of different workers on the interval between calving and first post-partum oestrus in buffaloes, which varied from 30 to 118 days. The earliest interval which he recorded was 22 days. Services at these early post-partum oestrus were less effective. The intervals between calving and first post-partum oestrus and fertile oestrus were 16–76 days (average 43 days) and 22–282 days (average 81 days) respectively (Hafez, 1953). Shalash (1958) reported the first post-partum oestrus in 301 Egyptian buffaloes to be $141 \cdot 11 \pm 6 \cdot 1$ days. He also found the average number of artificial insemination/conception after calving in the animals as $1 \cdot 41 \pm 0 \cdot 015$. The best conception rate was observed by him at the interval of 51–100 days post-partum.

The post-partum oestrus in Murrah and grade Murrah buffaloes has been reported in India by Rao and Murari (1956) and Luktuke (1958, 1959). The average interval between calving and first oestrus was found to be 87 days in Murrah buffaloes. Luktuke (1959), however, found that buffaloes which calved during autumn and winter showed their first oestrus 55 days post-partum. This interval was 108 days for the animals calved during the spring. The corresponding intervals between calving and fertile heat were 85 and 155 days respectively.

MATERIAL AND METHODS

Observations on the post-partum oestrus were made in the experimental Murrah buffalo herd of the Division of Animal Genetics, Indian Veterinary Research Institute, Izatnagar. A total of 123 post-partum periods were closely observed during 1956 to 1960. The data pertain to buffalo-cows which were 7–14 years of age and had calved two to seven times. The oestrus in these animals was detected with the help of a young, active, vasectomised buffalo-bull. The reproductive organs of the animals were also examined regularly at two weekly intervals, six weeks after parturition, with a view to detecting the presence of an active corpus luteum and the occurrence of missed oestrus, if any. All the animals were served artificially during oestrus. Semen was extended by Kampschmidt's dilutor with the addition of sulphamezathine and antibiotic in doses recommended by Srivastava and Prabhu (1956). To ascertain the occurrence of ovulation at these oestrus the animals were examined within 10–12 days

post-service for the presence of corpus luteum. The intensity of oestrus was recorded as pronounced, normal and weak. The pregnancy was diagnosed clinically by rectal palpation method at 45–50 days after the service.

RESULTS

Out of 123 first post-partum oestrus studied, in 18 cases oestrus was induced by the enucleation of corpus luteum, as no heat was detected post-partum. The remaining 105 animals showed an interval of 115.58±7.1 days between parturition and first post-partum oestrus. The fertile heat in these animals appeared 149·36±7·4 days after parturition, i.e., 33·78 days on an average after the appearance of first post-partum oestrus. The average number of artificial inseminations required per pregnancy in these animals was 1.60. The details for each year are presented in Table I.

TABLE I. INTERVAL BETWEEN PARTURITION AND FIRST NATURAL OESTRUS, PARTURITION AND FERTILE OESTRUS AND NUMBER OF A.I. PER PREGNANCY

Year	No. of cases	Average interval between			No. of A. I. per pregnancy
		Parturition and first natural oestrus (days)	Parturition and fertile oestrus (days)	First natural oestrus and fertile oestrus (days)	
1956	16	116·56±13·2	159·81±19·0	42·25	1·56
1957	15	108·80±19·0	151·20±20·7	42·40	1·67
1958	24	157·37±22·1	175·87±22·7	18·50	1·54
1959	20	97·55±16·7	140·65±23·0	43·10	1·50
1960	30	97·06±11·4	127·46± 9·9	30·40	1·73
	105	115·58± 7·1	149·36± 7·4	33·78	1·60

The conception rate of first and subsequent services in 105 animals which exhibited natural post-partum oestrus are shown in Table II. It will be observed that 62 animals (59.00 per cent) conceived at first A.I. and 30 animals (28.6 per cent) at second A.I.

TABLE II. CONCEPTION RATE AT FIRST AND SUBSEQUENT POST-PARTUM OESTRUS

A. I. required	1	2	3	4	5 or more
No. of animals served	105	43	13	6	1
No. of conceptions	62	30	7	5	1
Percentage of conception	59·0	28·6	6·7	4·8	0·9

The incidence of the intensity of first post-partum oestrus is shown in Table III. It was found that the percentage of pronounced, normal and weak oestrus at first

post-partum heat was 1.9, 80.0 and 18.1 respectively. In six cases the oestrus was not followed by ovulation. The incidence of anovular oestrus worked out to be 5·7 per cent. In the 18 animals mentioned above, in which silent oestrus was recorded, oestrus was induced by enucleation of corpus luteum. The details pertaining to intensity of the induced oestrus and occurrence of ovulation are presented in Table III.

TABLE III. APPEARANCE OF FIRST POST-PARTUM OESTRUS (NATURAL AND INDUCED), ITS INTENSITY AND THE INCIDENCE OF ANOVULAR OESTRUS

	No. of animals	Percentage to the total No. of animals	Intensity of 1st post-partum oestrus						Incidence of anovular 1st post-partum oestrus	
			Pronounced		Normal		Weak			
			No.	%	No.	%	No.	%	No.	%
Animals in which natural 1st post-partum oestrus was observed	105	85·4	2	1·9	84	80·0	19	18·1	6	5·7
Animals in which 1st post-partum oestrus was induced by enucleation of corpus luteum	18	14·6	1	5·6	4	22·2	13	72·2	2	11·1
Total	123	100·0	3	2·5	88	71·5	32	26·0	8	6·5

The relation between the interval from calving to conception and the number of A.I. required are presented in Table IV. It will be seen from the Table that over 60 per cent of the animals settled within six months of parturition. There were only two animals which conceived within 30 days of calving.

TABLE IV. RELATION BETWEEN THE INTERVAL FROM PARTURITION TO CONCEPTION AND THE NUMBER OF A.I. REQUIRED FOR CONCEPTION

Post-partum interval to fertile services (days)	Total number of conceptions	Percentage of conceptions	Total No. of A.I. required	A.I./conception
Below 31	2	1·6	2	1·00
31–60	15	12·2	16	1·06
61–90	17	13·8	22	1·29
19–120	16	13·0	23	1·42
121–150	14	11·4	22	1·57
151–180	12	9·8	21	1·75
181–210	10	8·1	15	1·50
211–240	7	5·7	11	1·57
241–270	7	5·7	21	3·00
271–300	7	5·7	12	1·71
Above 300	16	13·0	36	2·25
—	123	100·0	201	1·63

DISCUSSION

In dairy cattle the interval from parturition to first post-partum oestrus ranges from 32–69 days. In Egyptian buffalo-cows this interval was reported by Hafez (1953) to vary from 30–118 days. In the present study the average interval from parturition to the appearance of first post-partum oestrus has been found to be 115 days and the interval to conception as 149 days. This means that the interval between appearance of first post-partum oestrus and conception was 34 days on an average. This finding is almost in agreement with that of Knapp (1956), who reported this interval as 36.6 days in Egyptian buffaloes.

No published reports are available on the incidence of anovular first post-partum oestrus in buffaloes. Hafez (1953), however, assumed that the low percentage of fertility in his experimental animals might have been due to anovular post-partum oestrus. In 5·7 per cent of the animals in the present study the first post-partum oestrus was not followed by ovulation. This observation is based on regular clinical examination of the animals for the occurrence of ovulation during mid-oestrous cycle.

A total of 201 inseminations was required for obtaining 123 pregnancies. When considering all inseminations including inseminations at anovular oestrus, the average number of A.I./pregnancy was found to be 1.70. The average number of services required per pregnancy in Egyptian buffaloes was 1.71 according to Hafez (1963) and 1.41 according to Shalash (1958). If the inseminations done at anovular heats in the present study are excluded from analysis, the number of A.I. required per conception works out to be 1.60.

The relation of post-partum breeding interval to reproductive efficiency has been studied by several workers in dairy cows (VanDemark and Salisbury, 1950; Edward, 1950; Shannon *et al.*, 1952; Beshlebnov, 1956a, b). Not much information is available on the optimum length of time after parturition at which a buffalo should be rebred. Hafez (1952) reported that the Egyptian buffalo-cows showed post-partum oestrus as early as 22 days after calving and these buffaloes took one to four services per conception. The low rate of conception at early post-partum oestrus according to him was due to unprepared endometrium for implantation of embryo. Shalash (1958) obtained the best conception rate in Egyptian buffaloes within 51–100 days after calving. It has been found in the present study that the shortest interval at which a buffalo conceived was 22 days. Best conception results have been obtained during post-partum interval from 61–120 days.

Low breeding efficiency in buffaloes is commonly encountered under field conditions, especially in areas where breeding is done by A.I. Weak oestrus, oestrus not followed by ovulation, and silent oestrus are to a large extent responsible for the low pregnancy results. Regular periodical clinical investigation of genitalia and detection of oestrus are necessary in order to raise the conception rate and shorten the calving interval.

SUMMARY

A study of post-partum oestrus in buffaloes of experimental Murrah herd of the Indian Veterinary Research Institute, Izatnagar, was made for a period of five years.

The interval between parturition and the appearance of first oestrus in 105 cases studied was $115 \cdot 58 \pm 7 \cdot 1$ days and the interval between parturition and fertile oestrus was $149.36 \pm 7 \cdot 4$ days. These animals required on an average $1 \cdot 60$ A.I./conception. The percentage of buffaloes which settled after first insemination was $59 \cdot 0$ and, after second, $28 \cdot 6$ only. The incidences of silent and anovular first post-partum oestrus were $14 \cdot 6$ and $6 \cdot 5$ per cent respectively.

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OBSERVATIONS ON AN OUTBREAK OF A DISEASE SIMILAR TO THE BRITISH MUCOSAL DISEASE IN A DAIRY FARM IN BOMBAY

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INSTANCES of an unusual condition affecting buffaloes and cattle have been reported from various countries including India. Olafson *et al.* (1946) reported a highly contagious disease of cattle in New York State and named it as 'Virus Diarrhoea, New York'. Ramsey and Chivers (1953) described a similar disease in Iowa and called the condition a 'Mucosal disease complex'. Pritchard *et al.* (1954) reported illness in Indiana cattle and labelled the condition as 'Virus Diarrhoea Indiana'. Hedstrom and Isakson (1951) reported similar condition in Sweden and named the same as 'Epizootic enteritis'. In England Dow *et al.* (1956) reported a similar condition and named it as British mucosal disease. Pande and Krishnamurthy (1956) published a report of a similar condition in India.

The clinico-pathological picture observed in these conditions is somewhat similar to the one observed in Rinderpest. There is evidence, however, to show that this is an altogether different condition with etiological agent different from Rinderpest.

Jarret (1958) observed that American mucosal disease has a low morbidity and high mortality rate, while Virus Diarrhoea-New York, and Virus Diarrhoea-Indiana have a high morbidity and low mortality and as such are similar to the British Mucosal Disease. Sapre (1962) while describing clinico-pathological picture of a condition similar to mucosal disease which he called as 'Ajanta Disease', observed low morbidity with a high mortality in affected animals.

The authors had an opportunity to investigate an outbreak of a condition similar to the British mucosal disease, in a private dairy farm in Bombay. The mortality encountered in the outbreak was low. The clinico-pathological diagnosis was confirmed by serological tests which were put by Dr. J. H. Darbyshire of Central Veterinary Laboratory, Weybridge, England. As these observations establish the prevalence of this condition it would be worthwhile to put on record these observations which are detailed in this paper.

MATERIAL AND METHODS

The study reported in this paper is based on the observation of an outbreak in a farm which consisted of 56 buffaloes, 15 buffalo-calves, one buffalo-bull and one cow.

All these animals were stabled in *pucca* cement concrete shade. The hygienic conditions maintained in the farm were fairly statisfactory. All the animals were protected by freeze-dried goat-tissue vaccine before a year or so. Exact date of vaccination was not available. Revaccination of all the healthy animals in the face of the present outbreak was undertaken to induce immunity against Rinderpest.

Autopsy findings reported in this paper were those observed in the post mortem examinations of case Nos. 223 and 227. Total W.B.C. counts reported were from case Nos. 224, 222, 188 and 197. For bacteriological examination blood at the height of temperature, tissues at autopsy and heart blood were used. For serological investigation sera samples, from 12 canvalescent cases, inactivated at 56°C for 30 minutes were used (Table I).

TABLE I. OBSERVATIONS ON AN OUTBREAK OF DISEASE SIMILAR TO THE BRITISH MUCOSAL DISEASE IN DAIRY FARM IN BOMBAY

(Table showing details of the sera samples and their results on Gel Diffusion tests)

Serial No.	Ref. No.	Course of disease (days)	Diarrhoea	Symptoms observed		abortion	Stage of collection of serum (days)	Results of Gel Diffusion tests
				Ulceration oral	skin lesion			
1	182 M	9	+	—	absent	aborted	10 (after recovery)	Negative
2	187 M	12	+	+	+	aborted	10	Positive
3	191 M	6	—	—	—	aborted	10	Positive
4	195 M	5	+	+	+	aborted	12	Negative
5	202 M	6	—	—	—	—	12	Positive
6	203 M	8	—	+	—	—	12	Positive
7	221 M	5	—	+	+	—	10	Negative
8	222 M	17	+	extensive	extensive	—	10	Negative
9	230 M	8	+	,,	,,	—	12	Negative
10	235 M	3	+	—	—	—	12	Negative
11	239 M	16	—	—	—	—	10	Negative
12	244 M	6	+	—	—	—	12	Negative

Laboratory animals: For biological test, swiss albino mice aged about six to eight weeks and locally bred rabbits obtained from the Animal House of the Bombay Veterinary College were used.

For experimental transmission a female buffalow-calf, aged about one year, passively immunized with anti-rinderpest serum was used. The serum in a dose of 20 ml. was injected 12 hours prior to inoculation of the suspected material.

Methods: Bacteriological and cultural examination was carried out by cultivating aerobically, anaerobically and microaerobically. Total W.B.C. count was carried by conventional method.

For experimental transmission the technique described by Dow *et al.* (1956) was used with slight modification. The spleen, lymph node, lungs and heart preserved

on ice were cut in small pieces asceptically and were ground with sterile alandum using ice-chilled, phosphate-buffered saline of pH 7.2. A 10 per cent suspension was prepared and was centrifuged at 3,000 r.p.m. for 30 minutes. The supernatent was collected and was treated with antibiotics—1,000 units of penicillin and 10 mg. of streptomycin per ml. For assessing the sterility, a few drops of the antibiotic treated suspension were added to broth. The sterile suspension was inoculated to the experimental calf. The experimental animal was kept under observation for 30 days.

3. Serological investigation was carried out in the Central Veterinary Laboratory, Weybridge, England, and Gel Diffusion tests were put for the study.

OBSERVATIONS

Clinical picture: The disease apparently started after the entry of two animals, freshly purchased, in the herd. These animals were purchased from an area from were no outbreak was reported. The animals were healthy and were in milk at the time of purchase.

The first symptoms were noted after eight days of the entry of these animals. The onset of the disease was generally marked by a sudden rise in temperature. There was a drop of 2°–3°F in this initial steep rise, after about 48 hours. In 20 per cent cases temperature ranged between 102° and 103° F; 64 per cent cases showed a rise of temperature between 103° and 105° F and 16 per cent showed a rise of temperature more than 105°F.

Diarrhoea was another common symptom. The faecal material was foul smelling and was admixtured with froth, mucus and in some severe cases with blood. In 5 per cent cases there was only mucus, while 22 per cent cases showed an admixture of mucus and blood. No diarrhoea could be observed in 28 per cent of the affected cases.

Cutaneous lesions and ulcerations in the oral mucosa were other prominent symptoms. The cutaneous lesions generally appeared on the third or the fourth day after the first thermal rise. In 20 per cent cases the lesions were severe and were seen at the interdigitital space, coronary band, under the tail, at the root of the horn and in some cases at the outer canthus of one or both the eyes. The lesions could be described as red, necrotic, eroded sore patches accompanied with peeling of the skin and being painful to touch. Scale formation, erosion and acute pain was particuarly marked at the interdigital space and coronary band, leading to lameness. Oral lesions in the form of necrotic, ulcerative patches could be observed at the lips, inside of the commissures, gums and at the dorsum of the tongue. Abortions were observed in 4 out of 19 affected buffaloes. No symptoms suggesting the involvement of the nervous system could be noticed in any of the affected animals.

Course of the disease: The course of the disease in 16 per cent of the cases was less than six days, in 60 per cent cases less than ten days and in 24 per cent cases more than ten days. The disease continued in the farm for about 40 days.

Morbidity and mortality percentage: Morbidity was 33.3 per cent and of these, the mortality was only 20 per cent. It may be observed that this mortality can be considered as a moderately low mortality incidence. The species wise morbidity and mortality figures are tabulated on the next page.

	Buffaloes	Buff. calves	Buff. bulls	Bulls	Cows
Total No. of animals	56	15	1	2	1
No. affected	19	4	1	1	..
No. recovered	14	4	1	1	..
No. died	5	..	..	..	..
Morbidity (%)	33·9	26·6	..	..	..
Mortality (%)	26·3	..	..	..	..
Total morbidity (%)	33·3				
Total mortality(%)	20				

Bacteriological, cultural and biological tests proved negative for any organisms of pathogenic significance.

Total W.B.C. count was ascertained in four cases and three of them showed leucopaenia. One showing 3,200 per c.mm. died on the seventh day of illness. Another showing 3,800 per c.mm. was positive on serological test for mucosal disease.

Autopsy findings: The oral cavity showed ulceration on gums. There was no extension of ulceration in oesophagus. In the thoracic cavity the lungs and heart appeared normal. In the abdominal cavity there was no appreciable change in rumen and reticulum. The small intestine showed extensive inflammatory changes with haemorrhages. Kidneys and spleen appeared congested. The brain appeared normal. No other organ showed any significant change.

Treatment: Treatment with sulphamezathine and penicillin was tried to combat secondary invaders (York, 1956). Symptomatic treatment with astringents and intestinal antiseptics as well as glucose saline intravenous was also given to the ailing animals. It was observed that the cases did not respond to the treatment, the disease running its full course. However, the low mortality is suggestive of reduced complications as a result of the treatment.

Experimental transmission: After 24 hours of inoculation there was a slight rise of temperature by 1°F, while on the third day the temperature fluctuated between 100° and 102·8°F. This temperature was maintained up to the fifth day, after which it showed a further rise to 103·2° F. This temperature persisted for three days more and started declining gradually. The thermal reaction persisted for 15 days.

Cutaneous lesions: Soreness of the skin was first marked on the fifth day of inoculation. This patch was first observed on the off fore, near the coronary band and the interdigital space. In next two days soreness and reddening of the skin could be observed on the sites mentioned above. Erosins of the skin were also observed at the neck region, base of the horn, ears and shoulder region. On the eighth post-inoculation day, the eruptions could be marked at the root of the tail, at the quarters and at the back. These lesions were painful to touch and extensive peeling was observed. These lesions healed gradually in next seven days. On the seventh post-inoculation day, a red eroded lesion developed at the upper gums. It was situated laterally above the right commisures.

Differential count: W.B.C. count was 3,600 per c.mm. on the fourth day, 3,000 per c.mm. on the fifth day and lowest, i.e., 2,800 per c.mm., on the sixth day. This leucopaenia is suggestive of a virus infection. W.B.C. count after the recovery of the experimental animal, i.e., on the 20th post-inoculation day was 5,200 c.mm. It appears, therefore, that leucopaenia could be observed during the maximum reaction in the experimental animals (Fig. 1).

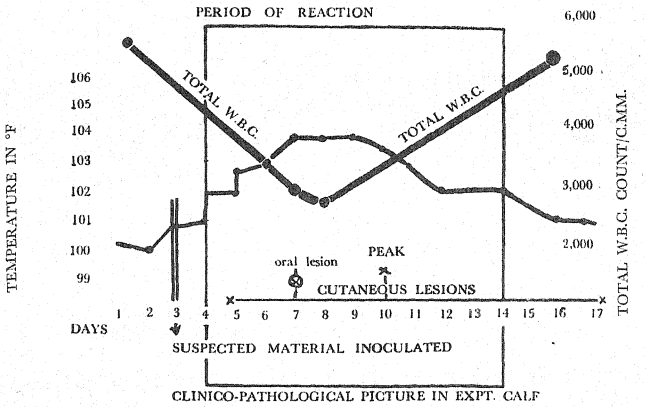

FIG. 1. GRAPH SHOWING THE FINDINGS AT THE EXPERIMENTAL TRANSMISSION

Even though the results detailed above are interesting, it is felt that critical host transmission experiments in many animals will only give us the exact nature of the capability of the casual agent to reproduce the disease and further studies on these lines are required.

Serological investigation: Sera samples from convalescent cases were forwarded to the Central Veterinary Research Laboratory, Weybridge, England. The details of these sera samples are tabulated in Table I.

The serological investigation showed that out of 12 sera samples, four proved positive on Gel Diffusion test for British mucosal disease (Derbyshire, 1962).

These serological tests confirm the clinico-pathological diagnosis of the disease as British mucosal disease.

DISCUSSION

The clinicopathological picture of the condition described in this paper is similar to the British mucosal disease described by Dow *et. al.* (1956). The diagnosis has been confirmed by the serological tests. Pyrexia, leucopaenia, cutaneous lesions, ulceration and abortion are the symptoms which are similar to the said condition. No cerebral

lesions including encephalitis were observed even though most of the affected cases were of buffaloes. The mortality rate was also low—only 20 per cent. It will thus be observed from the above, that the encephalitis and the high mortality described in recent outbreak by Sapre (1962) from Maharashtra State were not observed in the present study.

SUMMARY

Observations on an outbreak similar to the British mucosal disease reported from a farm in Bombay are detailed. Pyrexia, diarrhoea admixtured with blood and mucus, cutaneous lesions, oral ulcerations and abortions were the symptoms noted in the outbreak. Involvement of the central nervous system was not noticed. Haemorrhagic enteritis was the only prominent lesion noticed at autopsy. Leucopaenia was observed. Morbidity was 33·3 per cent, while mortality was only 20 per cent. The clinicopathological diagnosis was confirmed by Gel Diffusion test in which four out of 12 sera samples proved positive. Experimental transmission in a calf could produce a mild form of the disease with pyrexia, cutaneous lesions and leucopaenia.

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STUDIES ON PHAGE TYPING OF STRAINS OF *BRUCELLA* ISOLATED FROM HUMAN AND ANIMAL SOURCES IN INDIA

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DURING recent years phage lysis has been used extensively in the typing of *Salmonella, Shigella, Staphylococci* and *E. coli* strains in view of the specific nature of phage lysis, its comparative simplicity and reliability. Pickett and Nelson (1950) were the first to discover *Brucella* phages. Drozhewkina (1953) isolated a strain of *Brucella* phage which he and his co-workers (1955, 1956) used extensively in the study of Russian strains of *Brucella.* Parnas *et al.* (1958) isolated 14 phages from their own collection of 200 strains of *Brucella* and compared them with the Russian phage. Subsequent publications on phage typing of strains of *Brucella* from different sources (Parnas, 1961; Drimmelen, 1959, 1960; Stableforth and Morgan, 1958; Morgan *et al.*, 1960; Stinebring and Braun, 1959; Jones 1960) emphasize the need for studying the effect of these phages on *Brucella* strains isolated from different geographical regions and sources.

In the present study attempts have been made to study the effect of phages on strains of *Brucella* isolated and obtained from various parts of India, from cattle, buffaloes, human beings, goats and pigs including some of the type strains maintained in this laboratory, and to compare the results with those obtained on the basis of biochemical and serological tests generally employed in typing these organisms.

MATERIAL AND METHODS

Brucella strains: The following cultures of *Brucella* strains were employed in this study:

(i) Strain Nos. 616/47, 611, 10G/31 and 17G/28. These were recently isolated in this laboratory from Brucellosis-positive herd maintained at State Livestock Farm, Madhurikund.

(ii) Strain Nos. Hissar, *Br. suis, Br. melitensis*, F 49, V 19, F 37, F 31 and Bombay were received from the Director, Indian Veterinary Research Institute, Mukteswar-Kumaon.

(iii) Strain Nos. 8, 30, 45, 186, 341, 632, 799, 867, 887, 900, 924, 932, 939, 989, 997, B 2, B 36, G 11, AG, NL, SC, YPS, MP and 974 were received from Dr. T. N. Mathur, Assistant Bacteriologist, Karnal, Punjab.

(iv) Strain No. 12 bovis was received from Dr. Jozef Parnas, Lublin, Poland, which was the propagating strain used by him for *Brucella* phages isolated by him.

All the strains were streaked on Braun's agar plates, and after studying the morphology a single colony was picked up on Braun's agar slants. The usual biochemical and serological tests such as CO_2 dependence, H_2S production, sensitivity

to thionine and basic fuchsin, agglutination with high titre serum and monospecific sera, and smoothness of the colony were carried out simultaneously for all the strains. The procedure adopted at the Central Veterinary Laboratory, Weybridge, England, in 1953, was followed in conducting and interpreting these tests; the results are incorporated in Table IV.

Culture media: Braun's medium consisted of 1·4 per cent Agar powder (Difco) 1 per cent Dextrose and 3 per cent glycerol in 100 ml. of nutrient broth prepared from Lab. lemco and adjusted to pH 7·2.

(2) Tryptose broth was prepared by adding 2 per cent Bacto-tryptose (Difco), 0·5 per cent sodium chloride and 0·1 per cent Dextrose to 100 ml. of distilled water and pH adjusted to 7·2.

Propagation of phages: Nine phages, 212/XV, 371/XXIX, Tb, 24/II, 45/III, 10/I, 6, 7 and P, supplied by Dr. Parnas were used in this study. The phages were propagated by adding 0·6 ml. of the phage to 0·3 ml. of six-hour growth of *Brucella*-propagating strain (12 bovis) at 37°C in tryptose broth in small tubes of 1·2 cm. diameter and 10 cm. length. These tubes were kept in a water bath at 37°C for 15 minutes with occasional shaking, after which the material was transferred to a flask containing 25 ml. of tryptose broth. These flasks were kept at 4°C in the refrigerator for six hours and then transferred to an incubator at 37°C for 18 hours. This resulting suspension, either clear or turbid, was finally filtered through Seitz EK pads and was stored at —4°C. The phages were assayed for the potency before storing (Fig. 1).

Determination of routine test dilution (R.T.D.): Serial ten-fold dilutions of the phage suspension were made in a cold sterile physiological saline and were spotted with a small platinum loop in the middle of the fields seeded with six -hour-old tryptose broth cultures of the propagating strain on Braun's agar plate. One plate was used for one set of nine dilutions of the phage. The plates were incubated at 37°C for 18 hours. The highest dilution which produced confluent lysis was regarded as the routine test dilution (RTD) of the phage. The RTD was usually observed to be between 10^{-3} to 10^{-6} and no phage was used for typing purposes unless it had an RTD of at least 10^{-3}.

Phage typing: A six-hour-old culture of *Brucella* strain was seeded with a platinum loop at nine different fields on a plate of Braun's agar which was previously dried in the incubator at 37°C for 24 hours. The seeded plates were dried for 30 minutes in an incubator at 37°C and each *Brucella* phage was spotted over one field with the help of a small platinum loop. Each phage was used in both undiluted as well as at RTD. The results of phage lysis were interpreted by the presence of confluent lysis in the spotted fields of the culture (Fig. 2).

RESULTS

Titration of Brucella phage on propagating strain (12 *bovis*)*:* After the phages were received from Dr. Parnas, seven serial passages of all the nine phages were made on the propagating strain (12 bovis) supplied by him to boost up the rate of growth of the phages. The end titres of each phage on the propagating strain was assessed after these passages and the results of this titration were included in Table I. After these passages the end titres did not rise further and the RTD ranged between 10^{-3} and 10^{-8}.

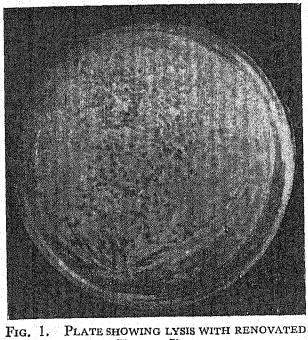

FIG. 1. PLATE SHOWING LYSIS WITH RENOVATED PHAGE (PHAGE 7).

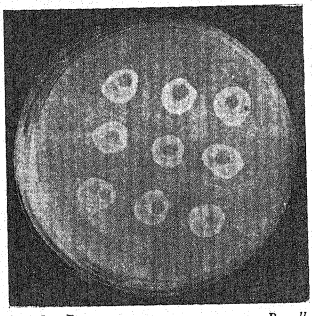

FIG. 2. PLATE SHOWING NINE FIELDS OF *Brucella* CULTURE WITH SERIAL DILUTIONS OF PHAGE 7. NOTE THE SPOT LYSIS WITH ALL THE DILUTIONS OF THE PHAGE.

These phage suspensions were used on the field strains for typing purposes and a titre one step lower than the RTD obtained during this titration was used for typing the *Brucella* strains.

Comparison of phage lysis test with undiluted phage and R.T.D. level: A comparative study of the lysis pattern of the nine phages on all the 37 strain of *Brucella* was made with undiluted phage and at RTD level; the results are incorporated in Table II. It would be evident from this Table that 24 out of 25 strains of *Br. abortus* were typable at both the levels of phage concentration. The one strain of *Br. abortus* which was not typable was a rough variant. All the three strains of *Br. abortus* isolated from buffaloes were typable by these phages. *Brucella* phage Nos. 7, 371/XXIX and 212/XV in general gave less active lysis on different strains as compared to the other phages at their R.T.D., although these phages exhibited high end titres. One of the six strains of *Br. melitensis* was typable by undiluted *Brucella* phages, while they were found to be ineffective at the R.T.D. level on that particular strain. Strain Nos. F 37 and F 31 of *Br. suis* were typable by all the phages excepting phage 212/XV. The phages showed slightly weaker lysis on these two strains as compared to those with *Br. abortus*-typable strains indicating differentiating pattern of lysis in *Br. suis*. The third strain of *Br. suis* was not typable.

Comparison of phage lysis with serological and cultural methods for species differentiation of Brucella: Common serological and cultural methods used for species differentiation of *Brucella* were carried out for all the 37 strains and the results of these tests were compared with those obtained by phage lysis (Table IV). It would be evident from the above Table that 24 out of 25 strains of *Br. abortus*, one strain of *Br. melitensis* and two of *Br. suis* were lysed by the *Brucella* phages out of 37 strains examined. The one non-typable strain of *Br. abortus* (17G/28) was observed to be a rough variant by acriflavine test as well as by colony characters, thus confirming the previous findings (Jones, 1960) that rough variants of *Br. abortus* are no longer lysed by *Brucella* phages.

TABLE I. TITRATION OF *BRUCELLA* PHAGES ON PROPAGATING STRAIN (12 BOVIS)

Phages	Phage dilutions									Routine test dilution (R.T.D.)
	Undiluted	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	
10/I	++++	++++	++++	++++	++	++	—	—	—	10^{-3}
P	++++	++++	++++	++++	++++	++++	+++	++	—	10^{-5}
45/III	++++	++++	++++	++++	++++	+++	+	—	—	10^{-4}
24/II	++++	++++	++++	++++	++++	++	++	+	—	10^{-4}
Tb	++++	++++	++++	++++	+++	+	—	—	—	10^{-3}
6	++++	++++	++++	++++	+++	+	—	—	—	10^{-3}
7	++++	++++	++++	++++	++++	++++	++++	++++	++++	10^{-8}
371/XXIX	++++	++++	++++	++++	++++	++++	++++	+++	+++	10^{-6}
212/XV	++++	++++	++++	++++	++++	++++	++++	+++	+++	10^{-6}

Index:

++++ = Confluent lysis
+++ = Plaques and spots
++ = Spots
+ = Less than 5 spots
— = No phage lysis

TABLE II. SUSCEPTIBILITY OF FIELD STRAINS OF *BRUCELLA* TO UNDILUTED AND ROUTINE TEST DILUTIONS OF *BRUCELLA* PHAGES

Strain of *Brucella*	Origin of cultures		Lysis by strains of *Brucella* phages																	
	Hosts	Source	10/I		P		45/III		24/II		Tb		6		7		371		212/XII	
			Undiluted	10^{-2}	Undiluted	10^{-4}	Undiluted	10^{-3}	Undiluted	10^{-3}	Undiluted	10^{-2}	Undiluted	10^{-2}	Undiluted	10^{-7}	Undiluted	10^{-5}	Undiluted	10^{-5}
616/47	Cow	U.P.	+	+	+	+	+	+	+	+	+	+	+	+	+	+(s)	+	+	+	+
611	Cow	U.P.	+	+	+	+	+	+	+	+	+	+	+	+	+	—	+	+	+	+(s)
10G/31	Buffalo	U.P.	+	+	+	+	+	+	+	+	+	+	+	+	+	+(s)	+	+	+	+
17G/28	Cow	U.P.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Hissar	—	I.V.R.I.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Br. melitensis	—	I.V.R.I.	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—
Br. suis	—	I.V.R.I.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
F 49	—	I.V.R.I.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
V 19	—	I.V.R.I.	+	+	+	+	+	+	+	+	+	+	+	+	+	+(s)	+	+	+(s)	+(s)
F 37	—	I.V.R.I.	+	+(s)	+	+	+	+	+	+	+	+	+(s)	+	+(s)	+(s)	+	+s	—	s
F 31	—	I.V.R.I.	+	+s	+	+(s)	+	+(s)	+	+(s)	+	+	+(s)	+	+(s)	—	+	+(s)	—	—
Bombay	—	I.V.R.I.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	Cow	Punjab	+	+	+	+	+	+	+	+	+	+	+	+	+	+(s)	+	+	+	+(s)
30	Cow	Punjab	+	+	+	+	+	+	+	+	+	+	+	+	+	+(s)	+	+	+	+(s)
45	Cow	Punjab	+	+	+	+	+	+	+	+	+	+	+	+	+	+(s)	+	+	+	+(s)
186	Cow	Punjab	+	+	+	+	+	+	+	+	+	+	+	+	+	+(s)	+	+(s)	+	+(s)
341	Cow	Punjab	+	+	+	+	+	+	+	+	+	+	+	+	+	+(s)	+	+	+	+(s)
632	Cow	Punjab	+	+	+	+	+	+	+	+	+	+	+	+	+	+(s)	+	+	+	+(s)
799	Cow	Punjab	+	+	+	+	+	+	+	+	+	+	+	+	+	+(s)	+	+	+	+(s)

TABLE II. (*Concld.*)

Strain of *Brucella*	Origin of cultures		Lysis by strains of *Brucella* phages																					
	Hosts	Source	10/I		P		45/III		24/II		Tb		6		7		371		212/XII	
			Undiluted	10^{-2}	Undiluted	10^{-4}	Undiluted	10^{-3}	Undiluted	10^{-3}	Undiluted	10^{-2}	Undiluted	10^{-2}	Undiluted	10^{-7}	Undiluted	10^{-5}	Undiluted	10^{-5}
867	Cow	Punjab	+	+	+	+	+	+	+	+	+	+	+	+	+	+(s)	+	+	+	+(s)
887	Cow	Punjab	+	+	+	+	+	+	+	+	+	+	+	+	+	+(s)	+	+	+	+(s)
900	Cow	Punjab	+	+(s)	+	+	+	+	+	+	+	+	+	+	+	s	+	+(s)	+	+(s)
924	Cow	Punjab	+	+	+	+	+	+	+	+	+	+	+	+	+	+(s)	+	+	+	+(s)
932	Cow	Punjab	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
939	Cow	Punjab	+	+	+	+	+	+	+	+	+	+	+	+	+	+(s)	+	+	+	+(s)
989	Cow	Punjab	+	+	+	+	+	+	+	+	+	+	+	+	+	+(s)	+	+	+	+(s)
997	Cow	Punjab	+	+	+	+	+	+	+	+	+	+	+	+	+	+(s)	+	+	+	+(s)
B2	Buffalo	Punjab	+	+	+	+	+	+	+	+	+	+	+	+	+	+(s)	+	+	+	+(s)
B36	Buffalo	Punjab	+	+	+	+	+	+	+	+	+	+	+	+	+	+(s)	+	+	+	+(s)
G11	Goat	Punjab	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
AG	Human	Punjab	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
NL	Human	Punjab	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
SC	Human	Punjab	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
YPS	Human	Punjab	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
MP	Human	Punjab	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
974	Cow	Punjab	+	+	+	+	+	+	+	+	+	+	+	+	+	+(s)	+	+(s)	+	+(s)
12 bovis	Cow	Poland	+	+	+	+	+	+	+	+	+	+	+	+	+	+(s)	+	+(s)	+	+(s)

Index: + =Confluent lysis; (s) =More than 20 spots; s =Less than 20 spots; — =No phage lysis

TABLE III. FREQUENCY OF LYSIS BY PHAGES UPON 37 STRAINS OF *BRUCELLA* (*BR. ABORTUS* 25, *BR. MELITENSIS*, 6 *BR. SUIS* 3, AND VARIANTS 3)

Phages	Dilution of phages	Number of strains lysed			
		Br. abortus	*Br. melitensis*	*Br. suis*	Variants
10/I	Undiluted	24	1	2	..
	R.T.D.	24	..	2	..
P	Undiluted	24	1	2	..
	R.T.D.	24	..	2	..
45/III	Undiluted	24	1	2	..
	R.T.D.	24	..	2	..
24/II	Undiluted	24	1	2	..
	R.T.D.	24	..	2	..
Tb	Undiluted	24	1	2	..
	R.T.D.	24	..	2	..
6	Undiluted	24	1	2	..
	R.T.D.	24	..	2	..
7	Undiluted	24	1	2	..
	R.T.D.	24	..	1	..
371/XXIX	Undiluted	24	1	2	..
	R.T.D.	24	..	2	..
212/XV	Undiluted	24	1	..	..
	R.T.D.	24	..	1	..

Strain Nos. AG, YPS and MP showing biochemical characters of *Br. melitensis* and serological characters of *Br. abortus* [which Renoux (1952) has named as *Br. intermedia*] have been recorded out of the cultures received from the Punjab. These strains were from human sources and were found to be resistant to phage lysis.

Five of the six strains having the serological and cultural characters of *Br. melitensis* were resistant to phage lysis. Out of the three strains which showed serological and cultural characters of *Br. suis*, two were differentiated from the third one by phage lysis.

DISCUSSION

The discovery of *Brucella* phages has led to the use of phage lysis as a valuable method for taxonomic classification and for epizootiological studies. In India so far no systematic work has been carried to study the use of phage lysis on the local strains

TABLE IV. COMPARISON OF PHAGE TYPING WITH STANDARD METHODS OF SPECIES DIFFERENTIATION IN *BRUCELLA*

Strain of *Brucella*	Need for CO_2	H_2S production (in days)	Smooth (S) or Rough (R)	Growth in the presence of		Agglutination in high titre serum	Agglutination in monospecific serum		Species or variant	Lysis by phage	
				Basic fuchsin	Thionin		*abortus*	*melitensis*		Undiluted	Routine test dilution
616/47	+	2 to 3	S	+	—	+	+	—	*Br. abortus*	+	+
611	—	1 to 3	S	+	—	+	+	—	—do—	+	+
10G/31	+	1 to 3	S	+	—	+	+	—	—do—	+	+
17G/28	—	1 to 3	R	+	—	+	+	—	—do—	—	—
Hissar	—	None	S	+	+	+	—	+	*Br. melitensis*	—	—
Br. melitensis	—	None	R	+	+	+	—	+	—do—	+	—
Br. suis	—	4 to 5	S	—	+	+	+	—	*Br. suis*	—	—
F 49	—	None	S	+	+	+	—	+	*Br. melitensis*	—	—
V 19	—	4	S	+	—	+	+	—	*Br. abortus*	+	+
F 37	—	4 to 5	S	—	+	+	+	—	*Br. suis*	+	+
F 31	—	4 to 5	S	—	+	+	+	—	*Br. suis*	+	+
Bombay	+	2 to 3	S	+	—	+	+	—	*Br. abortus*	+	+
8	+	3	S	+	—	+	+	—	—do—	+	+
30	+	2 to 3	S	+	—	+	+	—	—do—	+	+
45	+	2 to 3	S	+	—	+	+	—	—do—	+	+
186	+	2 to 3	S	+	—	+	+	—	—do—	+	+
341	+	2 to 3	S	+	—	+	+	—	—do—	+	+
632	+	2 to 3	S	+	—	+	+	—	—do—	+	+
799	+	2 to 3	S	+	—	+	+	—	—do—	+	+

TABLE IV—(*Concld.*)

Strain of *Brucella*	Need for CO_2	H_2S (in days) production	Smooth (S) or Rough (R)	Growth in the presence of		Agglutination in high titre serum	Agglutination in monospecific serum		Species or variant	Lysis by phage	
				Basic-fuchsin	Thionin		*abortus*	*Melitensis*		Undiluted	Routine test dilution
867	+	2 to 3	S	+	—	+	+	—	—do—	+	+
887	+	2 to 3	S	+	—	+	+	—	—do—	+	+
900	+	2 to 3	S	+	—	+	+	—	—do—	+	+
924	+	2 to 3	S	+	—	+	+	—	—do—	+	+
932	+	2 to 3	S	+	—	+	+	—	—do—	+	+
939	+	1 to 3	S	+	—	+	+	—	—do—	+	+
989	+	2 to 3	S	+	—	+	+	—	—do—	+	+
997	+	1 to 3	S	+	—	+	+	—	—do—	+	+
B2	+	1 to 3	S	+	—	+	+	—	—do—	+	+
B36	+	1 to 3	S	+	—	+	+	—	—do—	+	+
G 11	—	None	S	+	+	+	—	+	*Br. melitensis*	—	—
AG	—	None	S	+	+	+	+	—	*Br. melitensis* bio-chemically and *Br. abortus* serologically.	—	—
NL	—	None	R	+	+	+	—	+	*Br. melitensis*	—	—
SC	—	None or trace	S	+	+	+	—	+	—do—	—	—
YPS	—	None	S	+	+	+	+	—	*Br. melitensis* bio-chemically and *Br. abortus* serologically	—	—
MP	—	None	S	+	+	+	+	—	—do—	—	—
974	—	2 to 3	S	+	—	+	+	—	*Br. abortus*	+	+
12 bovis	—	3 to 4	S	+	—	+	+	—	—do—	+	+

of *Brucella*. Mathur (1963) recently recorded the results of typing of a few strains of *Brucella* isolated by him from milk samples with TB/L/S phage which were apparently carried out by Dr. Brinley Morgan, and reported that only strains of *Br. abortus* showed phage lysis but not strains of *Br. melitensis*. In the present study nine *Brucella* phages were obtained from Dr. Parnas and after renovating all the phages their end titres and RTD were assessed. Thirty-seven strains of *Brucella* isolated from different parts of India were typed with all the nine phages. As it is necessary to ascertain whether the phage should be used undiluted or at RTD or at both concentrations, a comparative study of phage lysis both undiluted and at RTD levels was carried out with all the phages on these strains. It was observed that RTD level did not add any additional information except that a few strains which were lysed with concentrated phages were not lysed at RTD level, indicating that the use of undiluted phage would be more sensitive than at RTD level. These findings are in concurrence with the work of Williams-Smith (1948), Edwards and Rippon (1953), Coles (1959) and Pargaonkar and Eisenstark (1962) on phage typing of staphylococci.

Parnas *et al.* (1958) observed that the Russian phage and the 14 phages isolated by them did not show any lysis with strains of *Br. melitensis* or *Br. suis*, and only strains of *Br. abortus* which conformed strictly to FAO/WHO strain 544 (Weybridge) were typable. Similar results were obtained by Stinebring and Braun (1959). But Stableforth and Morgan (1958) found that number of *Br. melitensis* strains isolated from cattle in England were lysed by the phages supplied by Dr. Parnas. Drimmelen (1960) reported that phages obtained from Dr. Stableforth lysed only strains of *Br. abortus* and not *Br. melitensis* or *Br. suis*, whereas a phage variant derived from that stock was shown to lyse *Br. suis* also. Jones (1960) could type 31 out of 38 cultures isolated from cattle and five strains from human sources by phage lysis. Although most of these strains had the serological and biochemical characters of *Br. abortus*, nine of the strains from cattle were agglutinated by monospecific *melitensis* serum. He could not type out by phage lysis the strains of *Br. suis* from pigs and human beings and *Br. neotomae*. Morgan *et al.* (1960) observed that all the strains of *Br. abortus* including those of type II Wilson were lysed but none of the four strains of *Br. suis* were typable. They observed that out of 33 strains of *Br. melitensis* isolated in Great Britain 24 were lysed by the phages. Jones (1960) recorded that the *melitensis*-like strains isolated from cattle in Britain as well as in the U.S.A. were different from *melitensis* strains found in the Mediterranean area and are similar to *Br. abortus* in their susceptibility to phage lysis. During the present study it was observed that all the smooth strains of *Br. abortus* showed phage lysis with all the nine phages both in undiluted and RTD levels. All the strains of *Br. abortus* isolated from buffaloes showed phage lysis. One rough strain of *Br. abortus* and one of *Br. melitensis* were no longer lysed by phages, confirming the previous observations of other workers (Stinebring and Braun, 1959; Parnas *et al.* 1958; Jones, 1960). One strain of *Br. melitensis* showed phage lysis with undiluted phages, and as the source of this strain was not available it might have been from bovine origin similar to those observed by Jones (1960). Polding (1947) described 26 strains of *Brucella* isolated from different parts of this country which were not true *Br. abortus* in that they grow both on thionin and fuchsin and antigenically not sharply differentiated as pure *abortus*, while two of them were distinctly *melitensis* type which he named

A/M types. Three strains showing biochemical characters of *Br. melitensis* and serological characters of *Br. abortus* (*Br. intermedia* Renoux, 1952) isolated from human sources from the Punjab were found to be resistant to phage lysis. It would therefore be interesting to study the phage lysis pattern of such aberrant strains when they are freshly isolated. These observations indicate that *Brucella* phage lysis is a valuable method for characterization of strains of *Brucella* and to study their epizootiological significance.

SUMMARY

Nine *Brucella* phages obtained from Dr. Parnas were employed for studying phage lysis pattern of 37 strains of *Brucella* isolated and obtained from different parts of India from cattle, buffaloes, human beings, goats and pigs. All the 24 smooth strains of *Br. abortus* showed phage lysis with all the nine phages both in undiluted and at RTD levels. One strain of *Br. melitensis* showed lysis with undiluted phages but not at RTD. The rough variants did not show any lysis. All the strains isolated from buffaloes showed phage lysis similar to bovine strains. Three strains showing biochemical characters of *Br. melitensis* and serologically *Br. abortus* were not lysed by the phages.

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HISTOPATHOLOGY AND HISTOCHEMISTRY OF *OESOPHAGOSTOMUM* NODULE OF SHEEP AND GOATS

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WEINBERG (1909) was the first to describe the histopathology of the disease caused by nodular worms in various species of apes. Thomas (1910) reported that in man the worms were present in the nodules along with giant cells and in some with calcifications. Hall (1920), Goodey (1926), Schwartz (1931) and Spindler (1933) described pathological changes in some domesticated animals. Monnig (1934) held that nodules were seen only in immune animals, and Fourie (1936) stated that toxins were responsible for signs, lesions and even death. Andrews and Maldonado (1942) and Gorden (1950) discussed the cause of nodule formation. Tewari and Iyer (1961) showed the presence of *Corynebacterium pyogenes*, *Staph. aureus*, *E. coli*, *Streptococcus* and *Pasteurella* in the nodules.

In this article the tissue reactions (histopathology) and study of normal and infected tissues by histochemical methods are described.

MATERIAL AND METHODS

The infected intestines were opened and the contents removed. The nodular portions were removed, washed in normal saline and fixed in 10 per cent formal saline or in Bouin's fluid. The intestines of two kids, one experimentally infected with 1,000 and another with 2,000 juveniles were also used; in these the nodules were up to the size of a pea, hard and caseated, and contained pus when examined fresh, but did not seem to contain any juveniles.

Serial sections of the nodules were taken and stained by the routine method using haematoxylin and eosin for histopathological studies.

For histochemical studies the following tests were performed as given by Lillie (1954), Culling (1957) and Pearse (1960): 1. *Carbohydrates*: Periodic-acid Schiff technique (PAS), Best's Carmine method and Hale's method for acid muco-polysaccharides; 2. *Proteins*: Millon reaction (Baker modification), Mercury-bromphenol blue method, Ninhydrin-Schiff method and DMAB–Nitrite method; 3. *Lipids*: Sudan black B method and Acetone Sudan black method; 4. *Calcium*: Von Kossa method; 5. *Amyloid*: Congo red method; 6. *Plasma cell*: Unna-Pappenheim (Methyl green—Pyronin) method; 7. *Pigments*: Chrome alum-haematoxylin method for lipofuscin, Long Ziehl-Neelsen method for acid fast lipofuscin, and Perls' method for haemosiderin; 8. *Connective tissue*: Fast green-Van Gieson method, Mallory's phosphotungstic acid method, Silver-impregnation method for reticulin, Verhoeff's method for elastic tissue, Taenzer-Unna acid-Orcein method for elastic tissue, and Allochrome connective tissue method.

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Bacteriological examination of the nodules: Pieces of intestine with nodules from sheep and goats were sent to the Research Officer, Bacteriology, for identification of bacteria, if present. The microtome sections of the nodules were stained by Giemsa and Gram stain.

OBSERVATIONS

Gross pathology

In heavy infections the wall of the intestine from the pylorus to the rectum was studded with the nodules, but generally more nodules were present in the caecum and colon. The mucosa and serosa overlying the nodules were thickened in some cases. The nodules were greyish or dull white, usually small, single or in confluent masses and hard and gritty to feel. These were difficult to incise, the core being compact and caseous.

Extra-intestinal lesions were also encountered in heavy infections. In the liver the lesions appeared as linear necrotic tracts under the Glisson's capsule containing the migrating juveniles. In the mesenteric lymph nodes the nodules were lodged in the cortex as discrete, greyish-white, multiple necrotic or caseous foci not unlike those of tuberculosis. In the omentum there were small but conspicuous and gritty tubercles.

Histopathology

Three types of lesions were encountered: (a) nodular lesions in the submucosa, (b) nodular lesions in the serosa, and (c) extra-intestinal lesions. Of these, the first two types were frequent.

Nodular lesions in the submucosa: The granulomatous foci were prominently seen in the submucosa and the two muscular layers in most cases. The juveniles were seen in the core or periphery of these granulomatous foci in some cases, but never in the mucosa or muscularis mucosae; in others, the empty tracks or necrotic and infiltrative changes were present.

The submucosal nodules varied in size from minute, submicroscopic aggregations of inflammatory cells or cellular detritus to large, expansive, haemorrhagic, necrotizing or caseous foci bounded by strands of young connective tissue, columns of small and large mononuclear cells and stray neutrophils. The smallest nodule was made up of a necrotic core of clumps of degenerate cells of uncertain identity with pyknotic nuclei, the cytoplasm having become homogeneous. The inflammatory cells in the periphery showed polymorphic nuclei and varying eosinophilia. In cases without eosinophilic infiltration in the adjoining stroma and in the lamina propria of the mucosa the degenerate cells in the necrotic core were easily identified as neutrophils. In other cases both eosinophils and neutrophils were present. The necrotic core was invariably surrounded by layers of lymphocytes, plasma cells and hypertrophied mesenchymal cells, some of which showed hyperchromatism, condensation of nuclear membrane, pyknosis and karyorrhexis of the nuclei.

In the medium-sized nodules the necrotic core was much wider and enclosed by pyknotic cells and surrounded and infiltrated by layers of lymphocytes, plasma cells and macrophages. The young fibrous tissue formed a distinct wall.

In the large nodules the core usually showed zones of small, multiple haemorrhages—

coagulative or caseation necrosis--surrounded by concentric layers of pyknotic cells, intact or partially degenerate, small and large mononuclear cells and young fibroblasts. The entire nodule was walled off by a considerable amount of hyperplastic connective tissue.

The development of the granulomas caused necrobiotic changes in the adjacent muscular tissue, muscularis mucosa and mucosa. The two muscular layers showed disruption of the muscle bundles, hyalinization of the fibres, proliferative and degenerative changes in the sarcolemmal nuclei and cellular infiltration in the endomysial and epimysial tissue. The sarcoplasm was granular in some of the affected muscle fibres. The cellular infiltration, chiefly with lymphocytes and plasma cells, in the interstitial tissue was sometimes so pronounced that the muscle fibres were overrun by these cells, and only masses of pyknotic cells were seen over the hyalinized fibres. Mostly the inner circular fibres showed similar changes, the outer longitudinal bundles being largely unaffected or showing only slight cellular infiltration. The blood vessels revealed marked hypertrophy and hyperplasia of the endothelial cells.

The submucosa showed marked stromal changes in addition to the necrotic nodules. The blood vessels were distended and markedly compressed and congested near the nodules. The capillaries were literally packed with hyperplastic endothelial cells. The stromal tissue stained dirty purple and the collogen fibres were oedematous and disrupted. There were also aggregations of lymphocytes and plasma cells.

The muscularis mucosa was reduced or absent where nodules showed a tendency to expand towards the mucosa. The muscle bundles were indistinct and their nuclei were compressed or present in wavy parallel columns.

The mucosa near the nodules was flattened, reduced in thickness, or lost where the nodule encroached, and markedly disorganized. The epithelium was disorientated and the epithelial cells vacuolated or flattened, sometimes with frayed borders. The crypts of Lieberkühn were effaced due to pressure from the necrotic nodules. The lamina propria was heavily infiltrated with small and large mononuclear cells, stray neutrophils and plasma cells, and occasionally with eosinophils. Sections of small intestine taken at the level of Peyer's patches and those of caecum and colon, showed degenerative or proliferative changes in the lymph follicles (Fig. 1), when these were near the necrotic nodules.

In advanced cases the nodules were necrotic or caseous, and were present close together in the submucosa or muscular layers. Due to prominent necrosis it was rather difficult to determine the sites of primary lodgement of the juveniles. In some nodules the necrotic core was an expansive structureless mass, staining uniformly pink, or caseous, and columns of lymphocytes and plasma cells were present between the inner layers of this granulomatous tissue. A few nodules showed small pools of blood bounded by masses of pyknotic cells and also the multinucleate giant cells.

The mucosa and the muscular layers were frequently involved resulting in ulceration or replacement of the muscle bundles by connective tissue.

Nodular lesions in the serosa: These nodules were more or less similar to those of the submucosal nodules. These were, however, prominently encapsulated and the granulomatous connective tissue usually extended to the outer longitudinal bundles of the muscle fibres (Fig. 2), which were almost entirely replaced by connective tissue.

The inner circular fibres showed degenerative changes associated with mechanical pressure. The mucous membrane of such nodules was haemorrhagic or necrotic, and intensely infiltrated by neutrophils, lymphocytes, plasma cells and large mononuclear cells. Eosinophilic infiltration was also common. These cells usually revealed varying degrees of regressive changes. Some encapsulated and caseated nodules were also seen, causing atrophy of the muscular layers and the adjacent tissues.

In some specimens groups of macrophages laden with orange to brown pigment of unknown nature were prominent in the necrotic tracks.

Extra-intestinal lesions: In the mesenteric lymph nodes the juveniles caused an intense inflammatory reaction, destruction of the lymphoidal cells, haemorrhage and necrosis of the parenchyma, which in some cases proceeded to caseation and calcification. The lesions were mostly confined to the cortex and were well encapsulated. No juveniles were, however, seen in the sections. The capsule of the lymphatic gland in the proximity of the lesions was considerably thickened and the lymph nodules were atrophied or converted into necrotic, purulent or caseous masses, surrounded by columns of neutrophils, small and large mononuclear cells and a well-developed fibrous capsule. The adjacent sinuses were engorged and showed hyperplasia of the littoral cells, with varying degree of necrobiotic changes. The intermediary and medullary sinuses often showed infiltration with mature and immature lymphocytes and macrophages. The necrotic foci when invaded by bacteria became purulent.

The liver showed multiple necrotic or haemorrhagic foci in the parenchyma. The encapsulated nodules showed changes due to coagulation or caseation necrosis and calcification. In the affected lobules the hepatic cords were disarranged, the sinusoids infiltrated with neutrophils and lymphocytes, and distended with blood; the hepatic cells adjacent to lesions revealed necrobiotic changes. The Kupffer cells were hypertrophied.

In the omentum granulomatous, necrotic or caseous lesions of various sizes were seen. The young lesions showed small accumulations of degenerate and pyknotic neutrophils surrounded by one or two layers of lymphocytes, plasma cells and macrophages. The older lesions were firmly encapsulated and were similar to those seen in the serosa, except that the juveniles were not seen. The perforation of the mesothelium was not observed. The submesothelial tissue, as well as the rest of the parenchyma, showed distension of blood vessels and mobilization of small and large mononuclear cells.

The histopathological changes in the nodules found in the liver were similar to those found in the intestine. It has been shown by previous workers that in heavy infections the juveniles escape from the serosa and are then arrested either in the mesenteric lymph glands, omentum or in other organs like liver, where they undergo caseation and calcification.

Histochemical studies

Carbohydrate: PERIODIC-ACID SCHIFF REACTION (PAS): (a) REGULAR PAS: Control tissue: The connective tissue muscles and the mucin in the goblet cells of the intestine gave a PAS positive reaction.

Infected tissue: As in normal tissue, except that the mucin gave a stronger reaction, the caseated mass of the nodule was slightly PAS positive.

(b) SALIVA PAS: Control and infected tissues: Similar results as in regular PAS test.

(c) BENZOYLATED PAS: Control and infected tissues: No reaction was observed.

BEST'S CARMINE TEST FOR GLYCOGEN: (a) REGULAR BEST'S CARMINE TEST: Contol tissue: The epithelial cells of the intestine gave negative reaction, and the muscular layers showed a faint positive reaction.

Infected tissue: All the structures were negative.

(b) SALIVA BEST'S CARMINE TEST: Control and infected tissues: All the structures were negative.

HALE'S TEST: Control tissue: The mucin in the goblet cells of the intestine gave a positive reaction.

Infected tissue: As in the control tissue but comparatively increased mucin production was noticed.

RESULTS: (i) There does not seem to be any glycogen present in the control and infected tissues of the intestine. (ii) An increase in mucin production was noticed in the intestine, containing nodules. (iii) A positive staining reaction with PAS test, which was saliva-fast, suggests that some carbohydrate-containing tissue element is present in the nodules.

Proteins: MILLION REACTION: Control tissue: A slight positive reaction was shown by all the layers of the intestine.

Infected tissue: The caseated mass of the nodule was strongly positive and the other structures were slightly positive as in the normal tissue.

MERCURY-BROMPHENOL BLUE METHOD: Control tissue: All the structures were negative except the longitudinal and circular muscle layers which gave a faint positive reaction. In some of the epithelial cells a positive reaction was seen, as evidenced by small blue granules. These could be the cells of Paneth.

Infected tissue: The caseated mass of the nodule was strongly positive, and the epithelial cells, muscularis mucosa and longitudinal muscle fibres were also positive.

NINHYDRIN-SCHIFF METHOD: In the infected tissue the caseated mass gave a faint positive reaction. A stronger staining reaction was given by the goblet cells, epithelial cells and fibrous tissue surrounding the nodule.

DMAB-NITRITE METHOD: Control tissue: All the structures of the intestine showed negative reaction, though a very faint blue staining was seen.

Infected tissue: The contents of the nodule were strongly positive and other structures were also positive, though not as strongly as the caseated mass.

Results: These tests indicate that the caseated mass of the nodules is proteinous in nature; tyrosine and tryptophane-containing proteins, and protein-containing reactive amino groups are present.

Lipids: For lipids the Acetone-Sudan Black and the Sudan Black B methods were employed, but the results were not satisfactory.

Calcium: VON KOSSA METHOD: The sections of infected intestine stained with haematoxylin and eosin showed dark blue staining areas in the caseated mass of the nodule, thus indicating the possibility of calcium being present. The Von Kossa test gave a very faint brown colouration in some intestinal nodules and a positive reaction in the lymph glands and liver (Fig. 3).

RESULTS: The test shows that a small amount of calcium is present in the nodules in the intestine; its amount is larger in the nodules of the lymph glands and the liver.

Amyloid: Congo red method was used for the tests. Results were of not much significance.

Plasma cells: Unna-Pappenheim (Methyl green-Pyronin) method was used. The older nodules in the intestine and the serosa showed the presence of plasma cells surrounding the caseated mass and the nodules in the mucosa and submucosa were severely infiltrated. A few plasma cells were invariably seen in the sections of the nodules from omentum, mesenteric lymph glands and the liver.

Pigments: The lipofuscin or haemosiderin pigments were absent, as indicated by chrome alum-haematoxylin method for lipofuscin, Long Ziehl-Neelsen method for acid fast lipofuscin, and Perls' method for haemosiderin.

Connective tissue: Six tests were performed to determine the nature of the connective tissue surrounding the caseated mass of the nodule. The results obtained are given in Table I.

FAST GREEN-VAN GIESON: In the tissue containing a nodule the collogen fibres were stained purple or red purple. These fibres surrounded the cyst completely, especially when the nodule was small (Fig. 4).

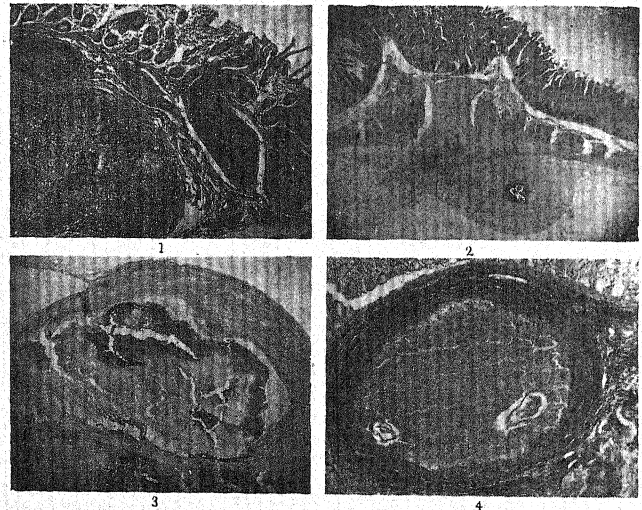

FIG. 1. INTESTINE, NODULE IN SUBMUCOSA. HAEMATOXYLIN EOSIN. *ca.* 13×
FIG. 2. INTESTINE, NODULE IN SEROSA. HAEMATOXYLIN EOSIN. *ca.* 13×
FIG. 3. LYMPH GLAND. VON KOSSA. *ca.* 25 ×
FIG. 4. INTESTINE, NODULE IN SUBMUCOSA. FAST GREEN-VAN GIESON. *ca.* 52×

TABLE I. NATURE OF THE CONNECTIVE TISSUE IN THE FIBROUS CAPSULE

Test	Result		
	Collagen	Reticular	Elastic
Fast Green-Van Gieson	Deep red purple	..	..
Mallory PTAH	Red brown	..	..
Silver-impregnation method	..	Black	..
Verhoeff's method	..	..	..
Taenzer-Unna Acid Orcein method	..	..	..
Allochrome connective tissue method	Bluish	..	..

MALLORY PTAH: In the tissue containing a nodule the collogen fibres were stained reddish brown. These fibres formed the cystic wall of the nodule.

SILVER-IMPREGNATION METHOD: With this method for reticulin some rather interesting observations were made. In the uninfected tissue the goblet cells of the epithelium showed deep staining, as if the mucus had been deeply impregnated and there was a network of black granules in other structures also. In the infected intestine, where the nodule was present in the submucosa, the goblet cells were not impregnated at all but the connective tissue around the nodules was impregnated and the other structures showed impregnation as in the uninfected tissue. In the infected tissue, where the nodule was found in the serosa, a few goblet cells were impregnated and the fibres surrounding the nodule were strongly impregnated.

VERHOEFF'S AND TAENZER-UNNA ACID ORCEIN METHODS: These methods gave unsatisfactory results.

ALLOCHROME CONNECTIVE TISSUE METHOD: In the normal tissue the striated border of the epithelial cells and the reticulin fibres present in the villi and the muscle fibres of submucosa, especially the longitudinal muscle fibres and the serosa, were stained bright blue. In infected tissue the connective tissue fibres surrounding the nodule stained bright blue, the basement membrane was almost completely obliterated and the circular muscle fibres were replaced by the reticulin fibres, though some discontinuous muscle fibres were seen.

The above results show that collogen is the main constituent of the fibrous nodule and the presence of reticular tissue and elastic tissue in the nodules is doubtful.

Bacterial flora of the nodules

The bacteriological examination of the nodular contents of several pieces of infected intestines showed the presence of Coli-aerogenes group of enteric bacteria, viz., *Escherichia coli*, *E. freundii* and *Clostridium welchii*. However, contradictory observations were made on the bacteria present in sections of nodules of the infected intestines. In Giemsa-stained sections sporulating rod-shaped organisms were invariably seen not only in the intestinal nodules but also in the omentum, mesenteric lymph glands and liver. Gram-stained sections did not reveal these bacteria.

DISCUSSION

Histopathological studies

The pathological findings reported in this article are largely in agreement with those reported by Hall (1920) and Fourie (1936). Necrotic nodules were also seen in the liver, mesenteric lymph nodes and omentum; similar extra-intestinal lesions were reported by several workers (Hall, 1920; Carne and Ross, 1932; Monnig, 1934; Sarles, 1944; Tewari and Iyer, 1961). According to Monnig (1934) the fourth-stage juveniles migrate to these organs through the blood or lymph channels. Fourie (1936) provided histological evidence that the nodules in these organs were the cemetery of the juveniles. A similar evidence was obtained in this study also.

Generally, the nodules were relatively numerous in the submucosa than in the muscular layers of serosa, and in no case there was primary involvement of the mucosa or muscularis mucosa. This may be due to the fact that the studies were carried out mostly in the aged subjects. Fourie (1936) demonstrated that the juveniles lodged themselves in the mucosa during the so-called 'primary parasitic migration phase', and that the muscularis mucosa is penetrated by the fourth-stage juveniles during the 'secondary abnormal parasitic migration phase'. It is rather difficult to speculate as to why the fourth-stage juveniles preferred to encyst in the submucosa. The vascularity and the texture of the stroma, which would permit easy penetration, may be the factors involved. However, the parasitic juveniles were also seen encysted in the muscle layers, although it was not possible, even in serial sections, to ascertain whether the parasites lodged themselves primarily in the muscles or in the interstitial connective tissue. Andrews and Maldonado (1942) observed that the parasitic juveniles were lodged in the blood vessels in the muscular coat in calves, but they did not indicate if a similar position obtains in the submucosal lesions also.

The microscopic appearance of the nodules varied markedly in the submucosa, where they were preponderant, principally due to the age of the lesions. That the lesions tend to be more severe in subjects previously exposed to infection was shown by Marotel (1908), Descazeaux (1926), Monnig (1934), and Andrews and Maldonado (1942). The early nodules were mere aggregations of inflammatory cells which were intensely pyknotic, but of undetermined nature. It is, however, reasonable to assume that where the lamina propria of the mucosa revealed infiltration with eosinophils, the inflammatory cells comprising the core of the nodules were also mostly eosinophils. Eosinophilic response in the mucosa and around the parasites is considered as an expression of the sound defence mechanism of the host (Monnig, 1934; Fourie, 1936). The presence of neutrophils could be attributed either to the degenerative changes in the nodules, or to the effect of the parasitic metabolites or to the presence of micro-organisms.

The periphery of the necrotic nodules, and also of the adjacent healthy stromal tissue, was densely infiltrated by lymphocytes, plasma cells and large mononuclear cells resembling macrophages. These cells are usually associated with chronic inflammation, and plasma cell response is believed to be intimately associated with antibody formation. It could not, however, be determined whether these cells were locally mobilized or transported through the blood stream from the lymphopoietic centres.

The nodules showed changes consequent to haemorrhage, coagulation or caseation necrosis. While haemorrhage may be considered incident to the migratory behaviour of the fourth-stage juveniles, coagulation necrosis may be ascribed to the liberation of metabolites by the juveniles. Feng (1931) believed that in *Physaloptera clausa* infection of hedgehogs and *Physaloptera caucasica* infection of monkeys the worms liberate a substance, which causes liquefaction of the tissues. Spindler (1933) thought that a similar process takes place in *O. longicaudum* infection in pigs.

The presence of the juveniles in the submucosa caused an intense inflammatory reaction, which gradually extended towards the muscular layers on one side and the mucosa on the other. The changes in the two muscular layers were characterized by cleavage of the muscle bundles, homogenization of the muscle fibres and marked round-cell infiltration. There was considerable hypertrophy and hyperplasia of the endothelial cells of the blood vessels in the muscular layers and the submucosa, possibly due to the absorption of the excretions—secretions of the parasite or the products of cell break-down. The expansion of necrosis towards the mucosa caused the dissolution of the muscularis mucosa and the ulceration of the mucosa proper. These ulcerating foci were found infiltrated with small and large mononuclear cells and the neutrophils and frequently by bacteria. The ulceration and perforation of the mucous and serous layers of the large intestine was due to the mechanical pressure from the juveniles.

There were also marked hyperplastic and degenerative changes in the lymph follicles of the caecum and colon and in the Peyer's patches of the small intestine. The hyperplasia of the reticular cells in the germ centres of these follicles and their metaplasia into mature and immature lymphocytes could be considered incidental to the absorption of the metabolites from the parasites or the products of cell-protein cleavage. The degenerative changes could be attributed to mechanical pressure. Only those follicles which were in proximity to the nodules were observed to show atrophic changes.

Fourie (1936) described that the lesions form cysts when present in the mucosa, but form nodules when present in the submucosa or other layers. After the third ecdysis inside the cyst the fourth-stage juveniles break through the mucosa and reach the intestinal lumen. This 'secondary parasitic migration phase' commences in about three to five days after infection (Fourie, 1936) or even earlier (Veglia, 1923). According to Fourie (1936), since the muscularis mucosa is a mechanical obstruction to the deeper penetration of the juveniles, normally the parasite is directed towards the lumen. But under certain unknown conditions the juveniles penetrate the muscularis mucosa and wander about in the deeper tissues. Monnig (1934) attributed this phenomenon to be due to immunity resulting from repeated infections.

The uninhabited cysts usually have a less well-developed fibrous tissue capsule than the cysts containing degenerate parasites. This may be due to the fact that the juveniles are able to break through the wall of the cysts only if the wall is comparatively thin. Alternatively, once the juveniles have escaped, there is no further host-tissue reaction and, therefore, the wall of the cysts remains thin.

Fourie (1936) observed that death in oesophagostomiasis was due to toxaemia and exhaustion, but did not provide any direct experimental evidence. Monnig and Fourie (cf. Fourie, 1936) inferred the presence of toxins purely on circumstantial

evidence. There were fatal cases in which relatively few nodules but numerous adult parasites were present and in which no complications such as enteritis were evident. In such cases emaciation, muscular atrophy and general cachexia appeared to be due to the toxic principles from the parasites themselves. They also discussed the possibility of the presence of toxic substances arising from the damaged mucosa.

In the present study extensive necrotic changes were observed in the wall of the small and large intestines, and parenchyma of liver and mesenteric lymph nodes; this supported the suggestion that, in addition to the parasitic toxins and end-products of cell destruction, the necrotic changes could bring about marked morbid effects which may lead to fatal consequences. This, however, requires further investigation and direct experimental proof.

Histochemical studies

Carbohydrates: The tests showed that some carbohydrate-containing tissue element is present in the caseated mass of the nodule, which is not glycogen. There was increased mucin secretion in the intestine due to the mild irritation caused by the presence of parasites.

Proteins: The tests showed that the contents of the caseated mass were chiefly made up of proteins, and that tyrosine and tryptophane are present. According to Munnich (1958) the glyco-protein increases with age due to the increased proliferation of the connective tissue and, also, the protein content remains unchanged, as shown by the intensity of the reaction. Later, he (1960) observed that ribonucleic acid increases with the beginning of regeneration of the destroyed tissue, outside the focus of inflammation.

In the present study the contents of the nodules gave positive reaction with the PAS and other protein tests suggesting the presence of glyco-protein; the protein contents increased in the infected intestine. However, an increase in the ribonucleic acid content of the surrounding tissue could be attributed to the increase in the number of plasma cells in the surrounding areas of the fibrous capsule.

Calcium: Calcification of the older nodules has been reported by Fourie (1936), and Mudaliar and Mudaliar (1942). Fourie (1936) recorded calcification as early as 28 days after the experimental infection, but some nodules did not show any evidence of calcification as late as 106 days. His findings were mainly based on the routine haematoxylin and eosin staining, in which calcified areas take up a deep blue stain.

In the present case the nodules from the intestine showed evidence of a slight calcification when sections were stained by Von Kossa method. The nodules from the mesenteric lymph nodes and liver showed a stronger reaction. This is probably due to the fact that calcification occurs around all dead and dying tissues which undergo a hyaline change and where the carbon dioxide tension is low. The calcium is also laid down around encapsulated dead parasites, which would happen to the juveniles reaching the mesenteric lymph nodes and the liver.

Plasma cells: Andrews and Maldonado (1942) recorded the presence of plasma cells in the early stage of multiple infection in calves. In the material used in this study the plasma cells were invariably seen in all the tissue components of the intestinal wall, mainly around the fibrous capsule of the nodule, mixed with other leucocytes and in

some cases, surrounding the parasite also. The mucosa was severely infiltrated with these cells in the later stages of infection.

The presence of numerous plasma cells comprises the most distinguishing feature of inflammation and their number helps in characterizing the acuteness or chronicity of the response (Robbins, 1957). They are believed to help in humoral defence mechanism of the host body by producing antibodies (globulins). A large number of plasma cells around the parasite and in the mucosa suggests that a mild type of toxin is secreted by the juveniles.

Pigments: The pigmentation of the tissues has been reported to occur in some helminthic infections, like schistosomiasis (Fairley, 1920), fascioloidiasis (Campbell, 1960) and dicrocoeliasis (Dhar and Singh, 1963). Apparently, there is no record of pigmentation in oesophagostomiasis.

In the material used during the experiment a light-brown pigment was present in the submucosa of the infected intestine. The pigment did not give the tests for lipofuscin and haemosiderin, and remains unidentified.

Connective tissue: The major constituent of the fibrous connective tissue is collogen and, sometimes, collogen fibres were seen mixed with the fibres of muscularis mucosa and circular muscle fibres of the intestinal wall. In oesophagostomiasis the irritant, whatever be its nature and origin, appears to be mild and which, acting for a considerably long time, produces chronic inflammation, resulting in proliferation of new tissue. When the irritant persists for a longer period, it stimulates the production of fibroblasts as a defensive mechanism adopted by the host tissue. According to Robbins (1957) these fibroblasts secrete a soluble pro-collogen that is precipitated within the mucopolysaccharide ground substance into insoluble collogen fibres.

The presence of reticular and elastic tissue could be regarded as doubtful, as the tests did not give satisfactory results.

Bacterial flora of the nodules

The secondary infection with bacteria has often been held as the cause of death in oesophagostomiasis, particularly when the ulceration of the mucosa and perforation of the serous surface are well marked. Fatal peritonitis has been known to ensue following perforation of the serosa (Theiler, 1921; Fourie, 1936). Weinberg (1909) stated that septicaemia produced by bacterial complications was the cause of death in apes having oesophagostomiasis, though he could not cultivate any bacteria. The work of Marotel (1908), Kinsley (1931), and Andrews and Maldonado (1942) indicates that bacteria have negligible role in causing the pathological changes in the intestinal wall.

In the present study sporulating, rod-shaped organisms were demonstrated in practically all the sections of not only the intestine but also of mesenteric lymph nodes, omentum and liver, supporting Cameron (1933) that bacterial contamination is very common. The micro-organisms were better visualized in sections stained with Giemsa rather than with Gram-stain, indicating that at least a few of them could be Gram-negative bacteria. Cultural examination of a few nodules selected at random revealed the presence of non-toxigenic type of *Clostridium welchii*, *Escherichia coli* and *E. freundii*. These organisms constitute the major components of the microbial flora of the intestinal

tract of healthy animals and it would seem reasonable to surmise that they were only accidentally carried by the migrating juveniles from the intestinal lumen into the intestinal wall and other organs. Further, the predominant neutrophilic cell-picture leads to the supposition that the micro-organisms may only be coincidental inhabitants of the nodules, playing no active role in their production. This conclusion is in conformity with the findings of Carne and Ross (1932) and Fourie (1936) in lambs and of Andrews and Maldonado (1942) in calves.

Several workers (Veglia, 1923; Fourie, 1936; Tewari and Iyer, 1961) reported fatal peritonitis as a result of perforation of intestinal wall and concomitant and consequent bacterial infection. *Corynebacterium pyogenes*, *Staphylococcus aureus*, *Streptococcus* sp., *Pasteurella* sp. and *E. coli* were isolated from the peritoneal fluid of goats showing lesions of peritonitis (Tewari and Iyer, 1961). Of these, only *Escherichia coli* was cultivated from the nodules present in sheep and goats in the present study. *Clostridium welchii* has been isolated, probably for the first time from the nodules, though Fourie (1936) had alluded to the occurrence of ' various types of bacilli having terminal spores '.

SUMMARY

The nodular lesions were more frequent in the caecum and colon, embedded in the submucosa in most cases. Similar lesions were also seen in the serosa, mesenteric lymph nodes, omentum and liver.

Histopathologically, three types of nodular lesions were seen. The submucosal lesions varied from submicroscopic aggregations of inflammatory cells to large necrotic and caseous masses surrounded by a well-defined granulomatous reaction.

Nodular lesions in the serosa, somewhat similar to those of the submucosal lesions, were firmly encapsulated. In heavily infected animals extra-intestinal lesions without parasites were seen. In the mesenteric lymph nodes the lesions were seen in the cortex as encapsulated granulomatous foci enclosing necrotic, caseous or calcified areas. In the liver there were areas of coagulation and caseation necrosis. Omentum showed lesions similar to those of submucosa.

The caseated mass of the nodule was found to be composed of glycoprotein but glycogen was absent. An increase in the mucin production was observed.

An unidentified pigment was found in the submucosa of the infected intestine.

A slight calcification was observed in the nodules of the intestine, but it was much more in the nodules in the mesenteric lymph glands and liver. Collogen formed the main constituent of the fibrous capsule and the presence of reticular fibres is doubtful. Plasma cells were invariably present around the nodule and the mucosa was found to be severely infiltrated by these cells.

Escherichia coli, E. freundii and non-toxigenic type of *Clostridium welchii* were isolated from the nodules. Sporulating, rod-shaped organisms were seen in the sections stained with Giemsa.

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EFFECTS OF THE AGE AT FIRST CALVING AND FIRST LACTATION MILK PRODUCTION ON LONGEVITY AND LIFETIME MILK PRODUCTION IN HARIANA CATTLE

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It has generally been observed that Indian cattle mature slowly (Sundaresan *et al.*, 1954). The opinion that early maturity might adversely affect the milk production in the first few lactations in Indian breeds of cattle, had been perhaps the main cause of cautious attempts to reduce the age at maturity. However, the effects of the age at first freshening on the milk yield in the first lactation were found to be non-significant in Hariana herds of Uttar Pradesh (Singh and Desai, 1961). An early age at first calving is expected to reduce the unproductive period of our cattle and so increase the lifetime millk production. Successful attempts have been made to reduce the age at first calving in Hariana cattle by breeding, selection, feeding, management and disease control at the District Dairy Demonstration Farm attached to the U.P. College of Veterinary Science and Animal Husbandry, Mathura. Hence the present study on this herd was taken up to find out the effects of the age at first calving and first lactation milk production on the longevity and the lifetime production, and whether the lifetime production could be increased by considering the age at first calving along with the first lactation production at the time of selection.

MATERIAL AND METHODS

The records of 100 foundation and 221 farm-bred Hariana cows were available at the District Dairy Demonstration Farm, Mathura for this study. Selected cows, 192 in number, were received from other state farms in 1947 and 1948. Complete records on only 100 selected foundation cows were available for this study. These animals had completed one or two lactations on other farms.

The 221 farm-bred cows were born, reared and completed at least one lactation since the inception of the farm up to June, 1962. So the records of the foundation and farm-bred cows were analysed separately.

The longevity was calculated in months from the date of birth to date of disposal and productive or useful life from age at first calving to the date of culling.

Cows are not kept in any herd until the end of their natural life and it is difficult to get a true measure of the lifetime milk production for the dairy cows. An arbitrary period was, therefore, selected for this study. The periods were selected as estimates of lifetime production: (a) production up to six years of age, (b) production up to eight years of age, (c) production up to ten years of age, and (d) production up to the age at culling.

The data used in the present study included age at first calving in months, milk yield in 305-day first lactation, milk produced in the first two 305-day lactations, milk

production in pounds up to six, eight and ten years and up to the age at culling and useful productive life and longevity in months. The effects of the age at first calving, first lactation yield on the latter characteristics were studied by simple and partial correlations and regressions. The multiple regression prediction equations were obtained as described by Snedecor (1946), the model being

$$\hat{Y}=\alpha+\beta\, y\ 1{\cdot}2\ (X_1-\bar{X}_1)+\beta y\ 2{\cdot}1\ (X_2-\bar{X}_2)+\epsilon$$

where α is the average of milk yield, β y 1·2 and β y 2·1 are partial regression coefficients, X_1, $\bar{X}_2$ are the age at first calving and milk yield in first 305-day lactation and ϵ is N $(0, \sigma)$.

RESULTS

The average of the age at first calving of 236* farm-bred heifers born during the period from 1947-48 to 1958-59 was 40·9±06·4 months. The youngest animal calved at the age of 26·1 months and the oldest at 85·3 months. The averages of the age at first calving according to year of birth, and percentage of animals saved and converted into cows each year are indicated in Fig. 1 to show the improvement brought about in age at first calving during the period of this study. Most of the culling of the young

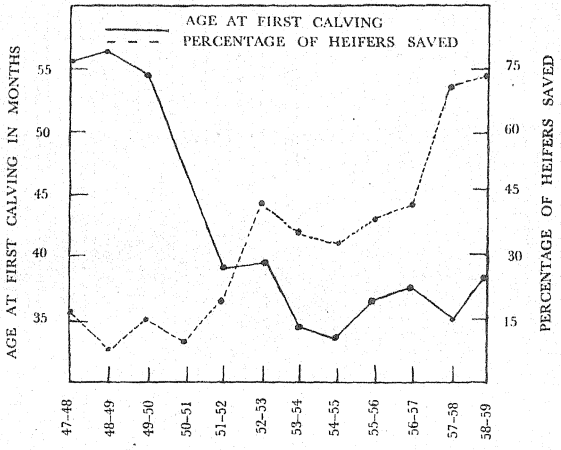

FIG. 1. AVERAGES OF AGE AT FIRST CALVING AND PERCENTAGE OF HEIFERS SAVED DURING DIFFERENT YEARS

heifer stock was done below the age of three years. In the year 1947–48 to 1951–52 76·3 per cent and in 1952–53 to 1958–59 99·4 per cent of the culled heifers were in

*Out of 236 only 221 completed the first lactation record.

this age limit. Only a few cases have been culled above the age of three years, 23·7 per cent in 1947–48 to 1951–52 and 0·6 per cent in 1952–53 to 1958–59.

The averages of the age at first calving, milk yield in 305-day first lactation, first two lactations, four different estimates of lifetime production, useful life and longevity of the foundation and farm-bred cows are presented in Table I. The average of age at first calving of the farm-bred cows decreased and the milk production up to ten years of age increased as compared to the foundation stock. The useful life, longevity and production up to the age at culling was lesser in the case of farm-bred cows than the foundation stock because the latter were retained for longer time than farm-bred stock, i.e., 48.6 months more. The coefficients of variation were found to be higher for each characteristic mentioned above in the farm-bred stock as compared to the foundation stock, suggesting further that the foundation stock was of highly selected ones.

Effect of age at first calving on the lifetime production, longevity and useful life: The coefficient of correlation and regression of the milk production in first as well as in the first two lactations on age at first calving were found to be non-significant (Tables II, III and IV).

The data on milk production up to six, eight and ten years of age were classified according to the age at first calving into 15 groups with a class interval of three months, the first group included animals up to 30 months of age and the last more than 69 months. The averages according to these groups have been presented in Fig. 2.

The coefficients of correlation of the age at first calving with the milk production up to six, eight and ten years of age were obtained as -0·758**, -0·582** and -0·640**, respectively, in the farm-bred cows, and -0·601**, -0·358** and -0·340**, respectively, in the foundation stock. The partial correlations of the age at first calving with milk yield up to six, eight and ten years when the milk yield in the first lactation was held constant in the same order were -0·860**, -0·715**, -0·662** in the farm-bred animals, and -0·915**, -0·382** and -0·358** in the foundation stock. The simple regression of the milk yield up to six, eight and ten years on the age at first calving in months were calculated to be -212·3**, -199·4** and -299·0** lb., respectively, in farm-bred stock. All these correlations and regressions have ben tabulated for comparison in Tables II, III and IV, and were found to be highly significant.

The coefficients of correlations of the age at first calving with milk production up to the age at culling, longevity and useful life were 0·320**, 0·233** and 0.326**, respectively, and when the milk yield in the first lactation was held constant they were 0·547**, 0·277** and 0·305**, respectively, in farm-bred cows. The regression of the milk production up to the age at culling and longevity in months on the age at first calving in months were 192·6 lb.** and 1·909** months respectively. These figures suggest that animals calving at higher age survived for longer period and so produced more milk. This finding reflects that weightage to the desirable extent on the age at first calving and on the total production up to the time of culling has not been given when selecting animals for replacement.

Effect of the first 305-day lactation milk yield on production of four periods of lifetime estimates, longevity and useful life: The records of the milk production up to six, eight and ten years

In this paper * and ** indicate statistical significance at 5 per cent ($P<0·05$) and 1 per cent ($P<0·01$) levels respectively.

TABLE I. AVERAGES OF AGE AT FIRST CALVING, MILK YIELD IN FIRST LACTATION FIRST TWO LACTATIONS, UP TO THE AGE OF SIX, EIGHT AND TEN YEARS, LONGEVITY AND USEFUL LIFE

Character	Foundation stock					Farm-bred					Overall				
	No.	Average	S.D.	S.E.	C.V.(%)	No.	Average	S.D.	S.E.	C.V.(%)	No.	Average	S.D.	S.E.	C.V.(%)
Age at first calving (in months)	100	55·1	8·3	0·83	15·0	221	40·1	9·5	0·64	23·6	321	44·8	11·4	0·63	25·5
Milk yield in first lactation (305 days; in lb.)	100	1609·0	727·4	72·40	45·2	221	2105·0	1072·0	72·10	50·9	321	1951·0	1003·0	55·90	51·5
Milk yield in first two lactations (in lb.)	100	3558·0	1350·0	135·00	37·9	164	4618·0	2016·0	157·00	43·7	264	3216·0	1863·0	114·60	57·9
Milk yield up to the age of 6 years (in lb.)	99	2106·0	1216·0	122·00	57·7	107	5902·0	3124·0	302·00	52·9	206	4078·0	3061·0	213·30	75·0
Milk yield up to the age of 8 years (in lb.)	92	4790·0	2127·0	222·00	44·4	63	10454·0	4035·0	508·00	38·6	155	7093·0	4125·0	331·30	58·2
Milk yield up to the age of 10 years (in lb.)	71	8055·0	2592·0	308·00	32·2	29	15393·0	5417·0	1001·00	35·2	100	10183·0	4924·0	492·40	48·4
Milk yield up to the age at culling (in lb.)	100	11429·0	7391·0	743·00	64·7	129	6426·0	5707·0	502·00	88·8	229	86·11	7335·0	485·70	85·3
Productive life (in months)	100	86·5	34·6	3·64	39·9	129	37·9	30·1	2·64	79·4	229	59·28	38·3	2·54	64·6
Longevity (in months)	100	141·6	31·2	3·15	22·03	129	79·5	35·2	3·10	44·3	229	106·60	45·4	3·00	42·58

TABLE II. CORRELATIONS OF THE AGE AT FIRST CALVING, MILK YIELD IN FIRST LACTATION, FIRST TWO LACTATIONS WITH LIFETIME PRODUCTION, LONGEVITY AND USEFUL LIFE

Character	No. of pairs		Age at first calving			Milk yield of first lactations			Milk yield of first two lactations		
	Foundation	Farm-bred	Foundation	Farm-bred	Overall	Foundation	Farm-bred	Overall	Foundation	Farm-bred	Overall
Milk yield in 305-day first lactation	100	221	-0·022	0·076	-0·097						
Milk yield in first two lactations	100	164	-0·004	0·123	-0·089	0·705**	0·893**	0·863**			
Milk yield up to six years	99	107	-0·601**	-0·758**	-0·782**	0·700**	0·861**	0·853**	0·449**	0·614**	0·674**
Milk yield up to eight years	92	63	-0·358**	-0·582**	-0·637**	0·670**	0·626**	0·763**	0·785**	0·672**	0·808**
Milk yield up to ten years	71	29	-0·340**	-0·640**	-0·534**	0·543**	0·765**	0·788**	0·688**	0·670**	0·696**
Milk yield up to age at culling	100	129	-0·094	0·320**	0·323**	0·418**	0·547**	0·391**			
Longevity	100	129	0·078**	0·233**	0·645**	0·395**	0·277**	0·120*			
Useful life	100	129	-0·086	0·326**	0·424**	0·360**	0·305**	0·150*			

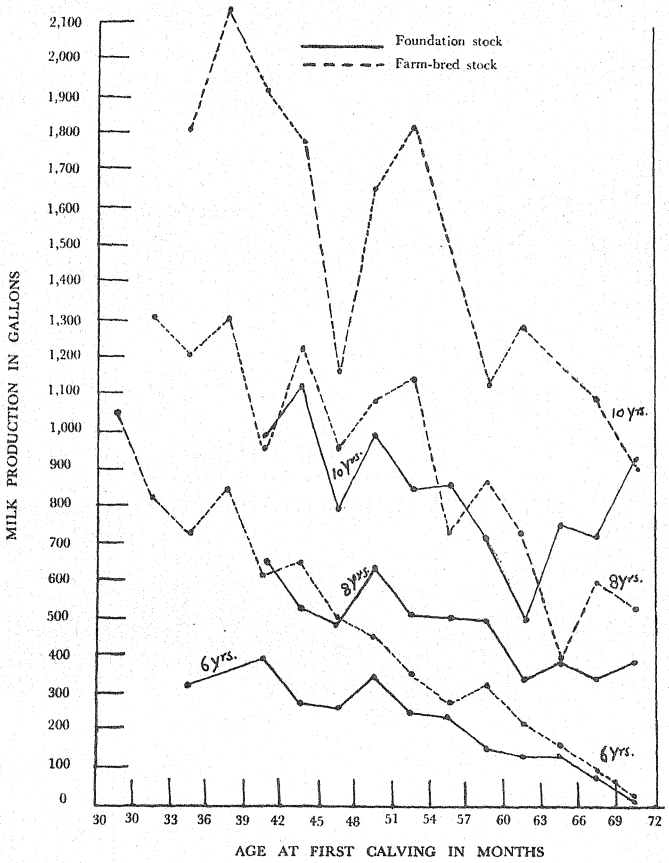

FIG. 2. RELATION OF AGE AT FIRST CALVING WITH MILK PRODUCTION UP TO THE AGE OF 6, 8 AND 10 YEARS

TABLE III. PARTIAL CORRELATIONS OF AGE AT FIRST CALVING WITH LIFETIME PRODUCTION WHEN MILK YIELD IN FIRST LACTATION WAS HELD CONSTANT AND THAT OF MILK YIELD IN FIRST LACTATION WHEN AGE AT FIRST CALVING WAS HELD CONSTANT

Correlation with	Partial correlation of age at first calving when milk yield was held constant			Partial correlation of milk yield when age at first calving was held constant		
	Foundation	Farm-bred	Overall	Foundation	Farm-bred	Overall
Milk yield in first two lactations	0·027	0·107	0·081	0·705**	0·892**	0·863**
Milk yield up to six years	–0·915**	–0·860**	–0·849**	0·886**	0·775**	0·813**
Milk yield up to eight years	–0·382**	–0·715**	–0·667**	0·677**	0·742**	0·777**
Milk yield up to ten years	–0·358**	–0·662**	–0·527**	0·557**	0·778**	0·804**
Milk yield up to age at culling	–0·093	0·350**	0·367**	0·422**	0·546**	0·432**
Longevity	0·094	0·229**	0·666**	0·399**	0·273**	0·204**
Productive life	–0·086	0·327**	0·436**	0·345**	0·307**	0·190**

of age were arranged, according to the milk yield in 305-day lactation, into 11 groups with the class interval of 200 lb. The first group included animals with production up to 1,200 lb. and the last group more than 3,000 lb. The averages according to these groups have been shown in Fig. 3.

Production during the first 305-day lactation was also correlated with the production in the first two lactations, four estimates of lifetime production, longevity and useful life (Table II). These correlations range between 0·547** to 0·893** in the case of lifetime estimates of milk production. The correlations with longevity and productive life were 0·277** and 0·305** respectively. The partial correlations of the milk production with these characteristics (Table III), when the age at first calving was held constant, were higher than the similar simple correlations shown in Table II. The linear regressions of the milk production in first two lactations, four lifetime estimates and longevity on milk yield in first lactation were highly significant as shown in Table IV.

Effect of milk yield in the first two 305-day lactations on production up to six, eight and ten years of age: The coefficients of correlation of the milk production in first two lactations with production up to six, eight and ten years of age were highly significant. These correlations when compared with similar correlations of first lactation with production up to six, eight and ten years suggest that accuracy for lifetime production shall not increase when production in the first two lactations was considered at the time of selection.

Prediction of lifetime production and longevity: Multiple regression equations for predicting production up to six, eight and ten years of age and production up to the age at culling and longevity were calculated by using the following three variables: (a) age at first calving in months X_1, (b) 305-day first lactation production (in lb.)

TABLE IV. SIMPLE REGRESSIONS OF THE MILK PRODUCTION DURING DIFFERENT PERIODS ON AGE AT FIRST CALVING, MILK YIELD IN FIRST LACTATION AND FIRST TWO LACTATIONS (IN FARM-BRED STOCK)

Regression of	No. of pairs	Regression on								
		Age at first calving			Milk yield in first lactation			Milk yield in first two lactations		
		b.	S.E.	t.	b.	S.E.	t.	b.	S.E.	t.
Milk yield in first lactation	221	8·579	7·960	1·079						
Milk yield in first 2 lactations	164	23·500	14·900	1·574	1·70	0·060	25·025**			
Milk yield up to 6 years	107	−212·300	18·200	−11·660**	1·88	0·245	7·960**	1·020	0·128	7·97**
Milk yield up to 8 years	63	−199·400	35·700	−5·590**	2·70	0·433	6·260**	1·611	0·215	7·48**
Milk yield up to 10 years	29	−299·000	69·170	−4·320**	4·91	0·790	6·430**	2·330	0·401	5·81**
Milk yield up to age at culling	129	192·560	50·910	3·780**	3·24	0·440	7·360**			
Longevity	129	1·909	0·230	8·268**	0·01	0·003	3·660**			

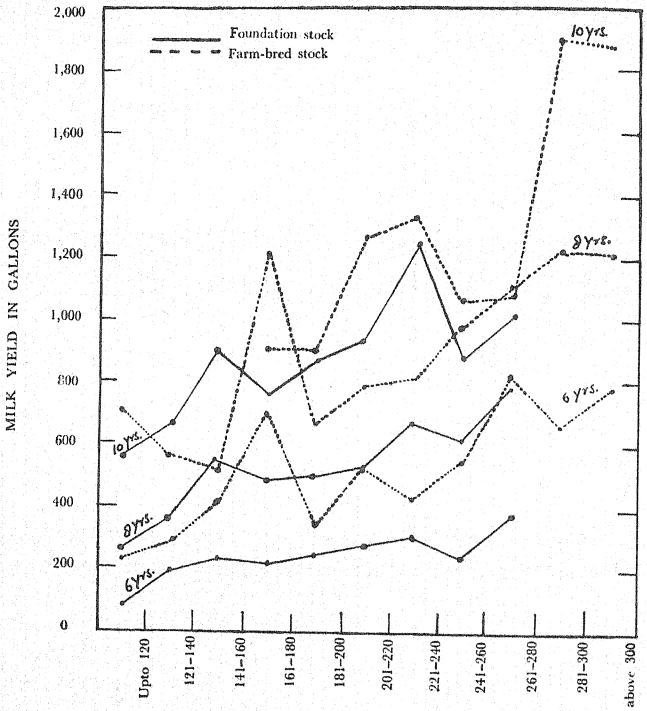

FIG. 3. RELATION OF FIRST LACTATION MILK YIELD WITH MILK PRODUCTION UP TO 6, 8 AND 10 YEARS OF AGE

X_2, and (c) production up to six, eight and ten years, production up to the age at culling and longevity in each case. These prediction equations are given on the next page, where Y is the predicted production in pounds, X_1 is age at first calving in months, X_2 is the milk production during the first 305-day lactation (in lb.). The square of the simple correlation coefficient between production in first lactation and production up to six, eight and ten years, age at culling and longevity are 0·741, 0·392, 0·585, 0.209 and 0·077, respectively, and are also shown in last column of prediction equations. The difference between R^2 and r^2 indicated that the addition of the

Production (in lb.) up to the age of		R	R^2	r^2
6 years	$\hat{Y} = 10386-193\cdot6X_1+1\cdot595X_2$	0·91	0·829	0·741
8 years	$\hat{Y} = 14600-191\cdot4X_1+1\cdot740X_2$	0·76	0·577	0·392
10 years	$\hat{Y} = 18925-205\cdot9X_1+4\cdot039X_2$	0·87	0·763	0·585
Culling	$\hat{Y} = -455-17\cdot71X_1+3\cdot168X_2$	0·54	0·294	0·209
Longevity in months	$\hat{Y} = 268+1\cdot88X_1+0\cdot139X_2$	0·55	0·306	0·077

variable age at first calving increased the accuracy of prediction in almost all the cases.

Heritability of longevity, productive life and production up to the age at culling: The heritability of longevity, useful life and production up to the age at culling could not be calculated either by intra-sire regression of the offspring on dam or half-sib correlation methods, as sufficient number of sires were not available and in certain cases the sires could not be determined. The heritability was, however, calculated by doubling the simple regression of daughter on dam by repeating the dam's record with each daughter's record (Kempthorne and Tandon, 1953). The heritability for longevity, productive life and milk production up to the age at culling were obtained as $0\cdot3794\pm0\cdot112$, $0\cdot3354\pm0\cdot114$ and $0\cdot296\pm0\cdot11$, respectively, on 101 dam-daughter pairs. In this study the records of eight dams were repeated thrice and that of another eight dams were repeated twice.

DISCUSSION

The concept that the reduction in age at first calving may adversely affect the production in the first few lactations could not find any support from the present findings, since neither first lactation production nor the first two lactation production was affected significantly by age at first calving. Similar results have been reported by Johansson and Hansson (1940), Gethin (1950), Robertson (1950), Sundaresan *et al.* (1954), Singh and Sinha (1960), and Singh and Desai (1961). However, Venkayya *et al.* (1956, 1957 a, b) obtained significant effects of age at first calving on the first lactation production.

The early calvers produced more milk up to the age of six, eight and ten years, presumably because of their longer productive periods they get as compared to late calvers. The findings observed here are in agreement with those of Chapman and Dickerson (1936), Sundaresan *et al.* (1954), Larson *et al.* (1951) and Singh (1963).

Late calvers survived selection, based on preceding lactation yield alone, for longer periods than the early calvers (Fig. 2), and so the late calvers had longer productive life and produced more milk during the period from age at first calving to the age at culling. This shows that due emphasis does not seem to have been given to the age at first calving or production up to the time of disposal at the time of selection. These findings, however, do not agree with those of Asker *et al.* (1954) and Singh and Sinha (1960), who obtained non-significant correlation between age at first calving and the longevity in Egyptian and Tharparkar cattle respectively.

Animals that produce more milk in the first 305-day lactation also produce more milk during the four estimates of lifetime production and survive selection longer than the low producers (Table I). This is borne out by the correlations given in Table II. Similar observations were made by Larson *et al.* (1951), Sundaresan *et al.* (1954) and Parker *et al.* (1960).

The findings, that the age at first calving and the 305-day first lactation milk production are not dependent on each other and that these two variables are significantly correlated with the lifetime production and longevity, supported the promises that they might be profitably included in multiple regression equations for predicting the lifetime production and longevity. The addition of the age at first calving increased the accuracy of prediction (obtained by difference of R^2–r^2) of milk production up to six, eight and ten years and longevity by 9, 19, 18 and 22 per cent, respectively, of the variance, which is a noticeable increase. It further emphasizes the need that age at first calving and the first lactation production should be considered as factors for improving the lifetime production. The findings noted above agree with those of Larson *et al.* (1951) and Sundaresan *et al.* (1954).

The heritability estimates obtained here for longevity, useful life and production up to age at culling (0·379, 0·335 and 0·296 respectively) suggest that improvement in these traits can be brought about effectively by selection. Similar estimates on dam-daughter regressions for the length of useful life and lifetime production were reported by Kaiser (1960) as 0·18 and 0·13 respectively; Wilcox *et al.* (1957) calculated heritability of longevity as 0·37. The estimates of Plowman and Gralaas (1960) for longevity were lower, i.e., 0·148 than what has been reported in the present study and the estimates of heritability of longevity reported by Parker *et al.* (1960) were extremely low.

It may be concluded from the discussion above that the existing method of selection on milk yield in the first lactation in case of replacement stock and on preceding lactation yield for those living herd does not seem to be accurate for increasing lifetime production and longevity. It needs to be supplemented by age at first calving as well as total milk production at the time of selection. This can be done by considering the production of per day of life from birth to disposal.

SUMMARY

The influence of the age at first calving, milk yield in first 305-day lactation on lifetime production, longevity and useful life have been investigated on the herd at the District Demonstration Dairy Farm attached to the U.P. College of Veterinary Science and Animal Husbandry, Mathura. The improvement brought about in the age at first calving at this Farm has been shown graphically. Four periods up to six, eight and ten years of age and up to the date of culling were chosen as estimates of lifetime milk production. Neither the first lactation production nor the first two lactation production were affected by the age at first calving. The early calvers produced more milk up to the age of six, eight and ten years. The late calvers survived selection for longer periods and so they had more productive life and produced more milk up to the age at culling. The animals that produce more milk in the first lactation also produce more during the four estimates of lifetime production and survive longer than the low

producer. The addition of the age at first calving with the production in the first lactation increased the accuracy of the prediction equations of milk production in latter life and longevity and, therefore, due emphasis should be given to the age at first calving and total milk production when selecting the replacement cows. The heritability estimates obtained here for longevity, productive life and milk production are quite high—0·379, 0·355 and 0·296, respectively—suggest that improvement by selection in these traits can be brought about. This can be done by considering the production of per day of life from birth to culling to increase the lifetime production.

ACKNOWLEDGEMENTS

Thanks are due to Shri C. V. G. Choudary for providing the facilities in conducting this work, and to Shri N. K. Hatwal, Farm Veterinary Assistant Surgeon, for his cooperation in supplying the data.

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THE INTESTINAL THREAD WORM, *STRONGYLOIDES PAPILLOSUS* (WEDL, 1856) RANSOM 1911, IN YOUNG BUFFALO-CALVES—A HISTOLOGICAL STUDY*

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U.P. College of Veterinary Science and Animal Husbandry, Mathura

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WHILE studying the pathogenic potentialities of this parasite in experimental infections in sheep, Turner (1959) reviewed the earlier work on this important species occurring in ruminants—domestic and wild. According to Woodhouse (1948), Mönnig (1938) considered that *S. papillosus* was not very pathogenic, especially in sheep, but Beveridge (1934), on the contrary, believed that the species played an important part in introducing the bacillus causing foot-rot. The clinical symptoms and the pathogenic effects, included under strongyloidiasis, associated with this and the other species of *Strongyloides* Grassi, 1879, are known in livestock including poultry, and have recently been found to involve the gastric mucosa in the kangaroo (Winter, 1953).

S. papillosus is widespread in our bovine, bubaline, caprine and ovine populations. Its incidence has been recorded by Vaidyanathan (1942), Sarwar (1945), Bhatia (1961), Patnaik and Pande (1963), and Sharma and Pande (1963). From Ceylon, Shanmugalingam and Serivertane (1954) recorded heavy infection predominantly in ten-day to eight-month-old buffalo-calves. Reference to lesions, both gross and microscopic, has also been made in some of these papers.

MATERIAL AND METHODS

In the 14 buffalo-calves (between one and three months in age), that were available for study, two belonging to Gynaecology Department had diarrhoea. In one of these calves, coccidian oocysts were also encountered. Faecal examinations, conducted before autopsy, revealed that four other calves were also positive for a pure infection with this parasite.

On post-mortem examination the duodenum and jejunum revealed distinct but small haemorrhagic patches and the intestinal contents included copious mucous exudate, with oedematous mucosal lining exhibiting irregularly raised and corrugated areas. In one case, however, very small nodules with a central reddish zone were visible. Scrapings from the former yielded characteristic eggs, usually in strings. Parasitic females were recovered from haemorrhagic patches. These two types of lesions were immediately preserved in 10 per cent formalin for subsequent histological study in order to assess the nature and extent of the pathological changes around the parasitic stages.

*Part of the thesis submitted by the senior author for M. V. Sc. degree of Agra University.

OBSERVATIONS

The mucosal patches of duodenum and jejunum exhibiting the characteristically diffused haemorrhagic spots yielded, in their serial sections, a picture of catarrhal enteritis. Strings of eggs were found in the superficial regions of the mucosa which was denuded (Fig. 1), the mucosal cells being desquamated with the surrounding area infiltrated with leucocytes, lymphocytes and a few eosinophils; the eosinophils predominating in the deeper regions of the lesions, while leucocytes and lymphocytes were observed in the vicinity of the egg masses (Fig. 2). A slight congestion of the blood vessels was also in evidence but there was no haemorrhage.

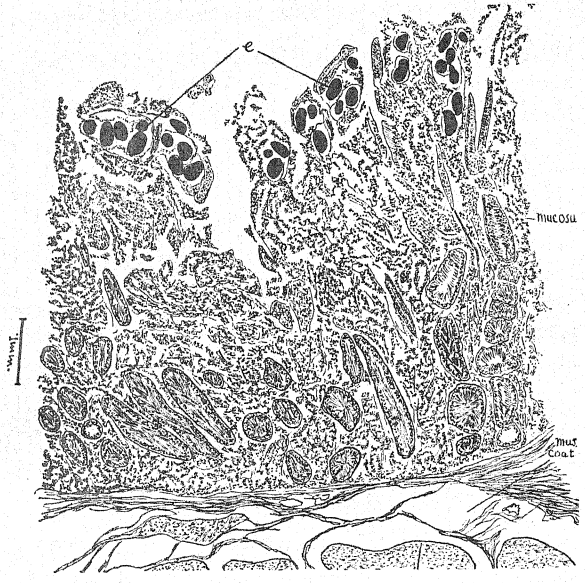
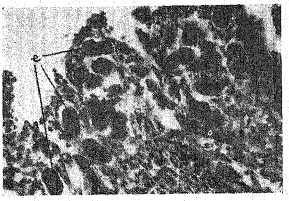


FIG. 1. SECTION OF INTESTINE WITH STRINGS OF EGGS (e) AND SUPERFICIAL MUCOSAL LAYER DENUDED (CAMERA LUCIDA DRAWING)

In the sections of the other type of lesions a parasitic female, in consequence of its tunnelling activity, was cut in different planes (Fig. 3) with the mucosa denuded. The degenerative changes including leucocytic and lymphocytic infiltration were visible all round (Fig. 4). Characteristic histology of the roundworm in these sections left no doubt about its indentity.



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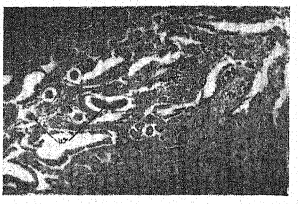

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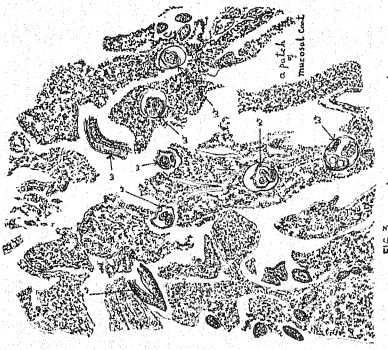

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FIG. 2. SECTION OF INTESTINE (PHOTOMICROGRAPH OF A PART OF FIG. 1) SHOWING EGGS (e) WITH REACTIONS AROUND THEM. × 150.

FIG. 3. PART OF ANOTHER SECTION WITH DENUDED AND DEGENERATED MUCOSA AND A WORM (w) CUT IN VARIOUS PLANES (CAMERA LUCIDA DRAWING).

FIG. 4. PHOTOMICROGRAPH OF A PART OF FIG. 3 SHOWING WORM (w) CUT AT VARIOUS PLANES WITH DEGENERATION OF MUCOSA AND INFILTRATION WITH MONONUCLEAR CELLS. × 85.

The small nodules, resembling the schizontic and other stages of coccidian parasites and whitish in character with a reddish tinge in the centre, were deeper in position, below the muscularis mucosae and somewhat spreading towards the muscular layer (Figs. 5, 6). In one of the series of such sections a female worm with eggs in uterus (Fig. 7) was cut. In consequence of the tunnelling activity, traumatic and lytic damage had been inflicted and a marked degree of fibroblastic activity was observed with lymphocytes, leucocytes and few eosinophils invading the area (Fig. 8). A slight degeneration of the underlying muscle bundles had also resulted. In such locations the mucosal lining exhibited a desquamation and an infiltration with reactionary cells.

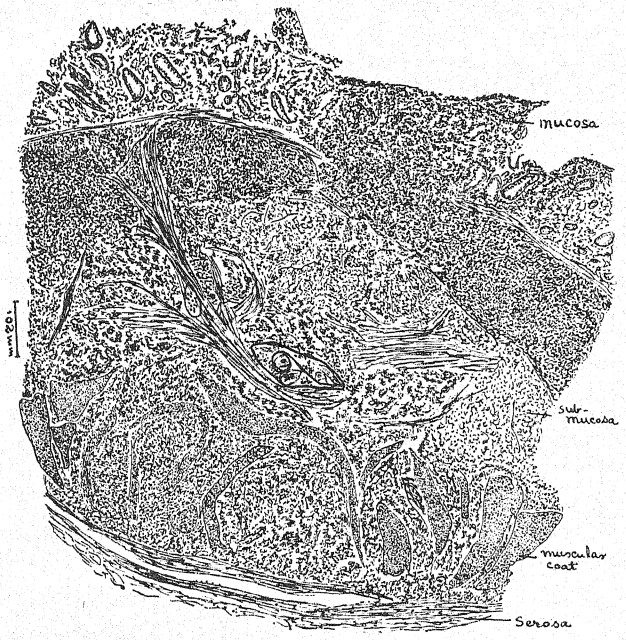

FIG. 5. SECTION OF THE NODULE (CAMERA LUCIDA DRAWING OF A PART; NOTE ITS SIZE, POSITION AND DAMAGE DONE TO MUSCULAR LAYER WITH FIBROBLASTIC ELEMENTS IN ITS CENTRE AND A DESQUAMATION OF MUCOSAL CELLS WITH A HEAVY INFILTRATION OF REACTIONARY CELLS).

In another series of sections of similar nodules, a phagocytic activity had involved the encapsulated worm, in which its internal structure had lost its normal feature and the body wall could alone be recognized (Fig. 9). The surrounding area revealed likewise an infiltration with lymphocytes and eosinophils in addition to a marked degree of fibrosis, all leading to the development of a ' pseudo-tubercle '. A complete degeneration of the deeper muscles was also evident and there was a complete denudation of the superficial mucosa.

DISCUSSION

The pathological changes in ' Strongyloidiasis ', from infections with *S. papillosus* in sheep, goats and in calves, have been described by Woodhouse (1948), Vegors and

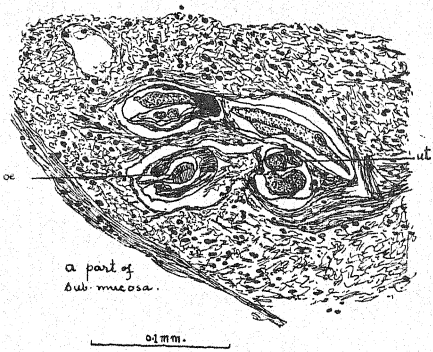

FIG. 6. SECTION OF NODULE (CAMERA LUCIDA DRAWING OF A PORTION OF FIG. 5) SHOWING THE OESOPHAGUS (oe) AND UTERUS (ut) OF WORM AND FIBROBLASTIC ELEMENTS WITH REACTIONARY CELLS.

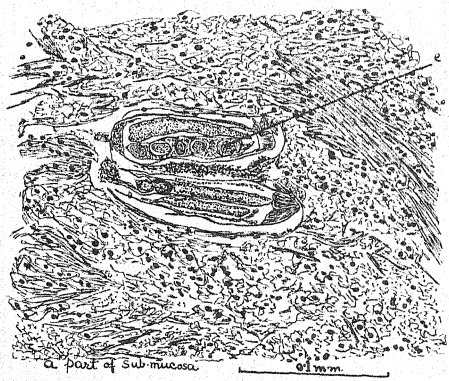

Fig. 7. SECTION OF NODULE (CAMERA LUCIDA DRAWING) SHOWING A FEMALE WITH EGGS(e) IN THE UTERUS AND FIBROBLASTIC ELEMENTS WITH REACTIONARY CELLS.

Porter (1950), Vegors (1954) and Turner (1959); details of the tissue damage, confined mostly to duodenum and jejunum, have also been given by these authors. Faust *et al.* (1934, 1934a) and Faust (1935), during experimental studies on species of *Strongyloides* and in dealing with the pathology of these infections, have referred to encapsulation

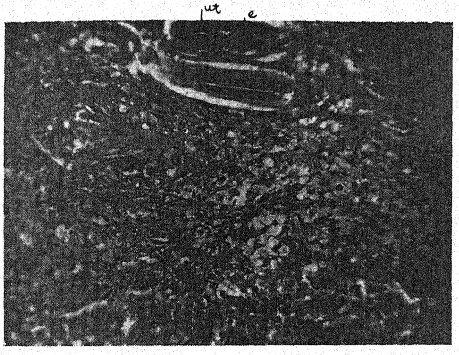

FIG. 8. SECTION OF A NODULE (PHOTOMICROGRAPH OF A PORTION OF FIG. 7) WITH UTERUS (ut) AND EGGS (e), AND FIBROBLASTIC CHANGES FROM REACTIONARY CELLS. × 150.

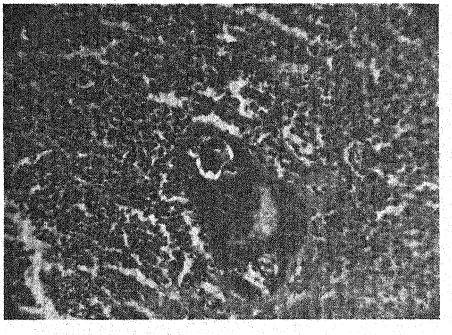

FIG. 9. SECTION OF NODULE SHOWING (PHOTOMICROGRAPH) TOTAL LOSS OF INTERNAL STRUCTURE OF A PHAGOCYTIZED WORM (NOTE FIBROSIS AND LYMPHOCYTIC AND EOSINOPHILIC INFILTRATION. × 150.

and phagocytosis encountered in response by the host tissue. Faust, observing pseudo-tubercles in muscularis mucosae invaded by larvae, stated that in all these local reactions the eosinophils were absent. The occurrence of juveniles of *S. papillosus* inside smaller-sized haemorrhagic nodules in duodenum has recently been reported by Sharma and Pande (1963). Both the sub-mucosal and muscular layers involved in this had revealed in their centre the developing stages with the adjacent blood vessels dilated and congested. Scattered haemorrhagic pockets were also stated to be in evidence.

The present study has shown the parasitic female in its tunnelling activity and the strings of eggs left by it. The pathological changes revealed in the normal infestations appear essentially similar to those described in experimental infections by Woodhouse, Turner and Vegors. The picture also resembles the pathogenic effects attributed to infections with such other species as *S. stercoralis* in dogs (Sandground, 1926), *S. ransomi* in pigs (Skalinski, 1953), *S. westerii* in donkey (Pande and Rai, 1960), and *S. avium* in chickens (Cram, 1929). The haemorrhagic nodules, reported by Sharma and Pande (1963), yielded pre-adult females localized inside. The present material, on the other hand, revealed more developed females (with eggs in its uterus) and in addition a pre-adult or an adult stage which, in consequence of its prolonged arrest in the encapsulated abode, had undergone phagocytosis. Such nodules evidently appear to have developed in consequence of the wandering pre-adult penetrating deeper into the sub-mucosal layers where, from host tissue reaction, encapsulation is accomplished—a condition recorded by Faust only in case of experimental infection in the dog.

SUMMARY

Strongyloides papillosus infection in buffalo calves, below three months of age, revealed gravid females during their tunnelling activity in the mucosal regions and the strings of eggs left by them in such areas. Pathological changes in and around such harboured stages have been described. Adult females, going astray in the submucosal layer, were found encapsulated in some sections with phagocytosis in another series.

ACKNOWLEDGEMENTS

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OCCURRENCE OF AORTIC ONCHOCERCIASIS AND SPIROCERCOSIS IN BUFFALO-CALVES WITH A NOTE ON THE REPORTS IN OTHER DOMESTIC ANIMALS*

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BULBOUS/THORACIC aortic infection with *Onchocerca armillata* Railliet and Henry, 1909, in bovines is known from a number of countries. It has recently been reported in Indian ovines also (Bhatia and Sood, 1959; Bhatia, 1960). The lesions in cattle and the morphology of this parasite have been described in detail by Chodnik (1957, 1958). Reporting cases of aortic affections in goat, pony and donkey from infestations with juveniles of *Spirocerca lupi* (Rud, 1809) Chitwood, 1930, Pande *et al.* (1961) indicated the possibility of its greater incidence in domestic animals in this country. Recently, in his investigations on aortic 'Onchocerciasis' in cattle in Orissa, Patnaik (1962) found this infection in 99 per cent of adult cattle with some bulls showing epileptiform fits and developing ocular complications, but the five young calves, between three and six months of age, did not reveal this infection.

MATERIAL AND METHODS

In the course of study on the common helminthic lesions of one- to six-month-old buffalo-calves (*Bubalus bubalis*), with special reference to those of three- to six-month-old, one of the ten calves in the latter group exhibited aortic lesions, which yielded a juvenile form of *Spirocerca lupi* that had attacked its intimal lining which was prominently damaged; in two others the characteristic onchocercal infection was found.

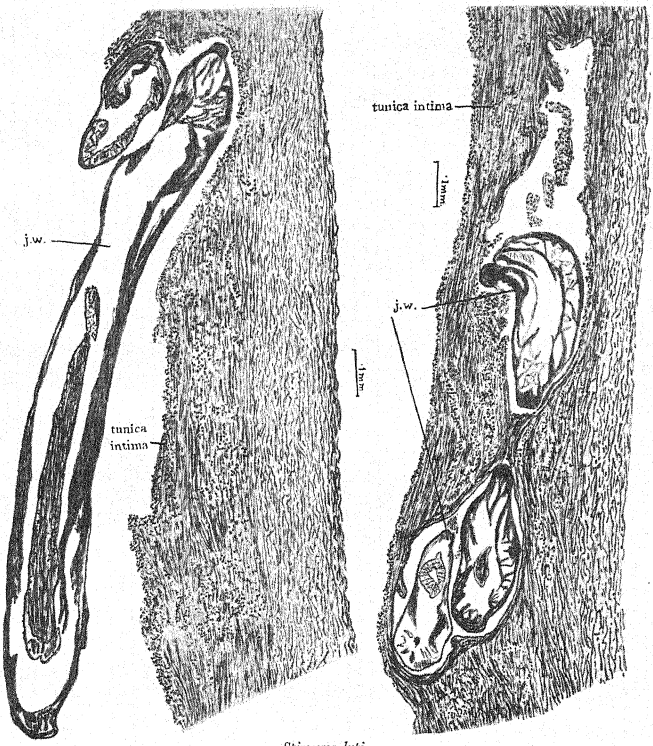
These two specific infestations, involved in the aortic onchocerciasis affecting nearly the same region, have been histologically studied from the affected material that was immediately fixed in 10 per cent formalin and serial sections, stained with haematoxylin and eosin. The lesions revealed important pathological changes in and around the harboured stages. Spirocercal juveniles, in later stages of development, from the aorta of an adult buffalo were also studied.

The present onchocercal infection in calves, from serially cut stained sections, was compared with similar material from buffalo, bullock and goat. The calves studied in this and the other group were negative for onchocercal infection of the ligament and subcutis.

*Part of the thesis submitted by S. C. Srivastava for M. V. Sc. degree of Agra University (1963) under the supervision of B. P. P.

RESULTS AND DISCUSSION

Spirocercosis: In consequence of this invasion both the endothelial lining and the intimal coat revealed prominent damage, consisting of complete denudation of endothelium at such spots, with the adjacent intimal coat partly destroyed (Fig. 1). In the area of the intimal coat harbouring the anterior part of the worm the evident pathological changes were focal areas of mononuclear cellular infiltration predominently lymphocytic with only a few macrophages and a very mild degree of fibroblastic activity.

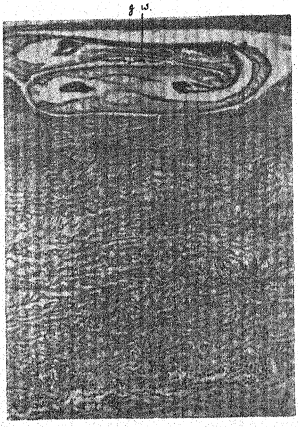


Spirocerca lupi

FIG. 1. SECTION OF AORTA SHOWING INTESTINAL PART OF JUVENILE OF *Spirocerca lupi* (j.w.) DAMAGING TUNICA INTIMA AND ENDOTHELIAL LINING OF AORTA (CAMERA LUCIDA DRAWING)

FIG. 2. SECTION OF AORTA SHOWING ANTERIOR END OF JUVENILE (j.w.) WITH DEGENERATION AND REACTIONARY CELLULAR INFILTRATION IN TUNICA INTIMA (CAMERA LUCIDA DRAWING)

The cellular elements, particularly those in close proximity to the parasite, revealed degenerative changes leading to necrosis (Fig. 2). The medial and the adventitious coats, in the serial sections, exhibited the smooth muscle cells and connective tissue elements as completely normal except for fibroblastic elements, which was suggestive of a mild degree of hyperplastic reaction (Figs. 3, 4), and a few mononuclear cells infiltrated in the medial layer. In the adventitia no abnormality was apparent.



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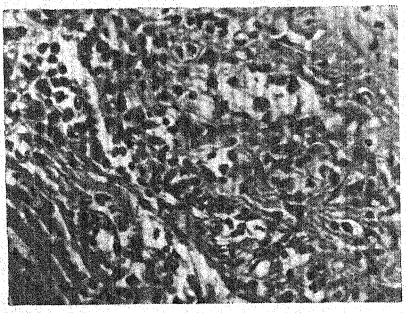

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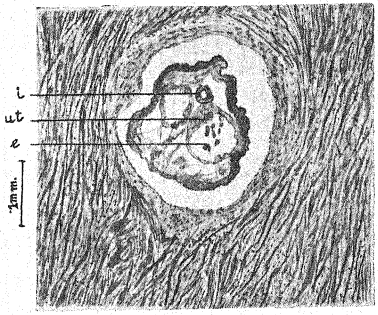
Spirocerca lupi

FIG. 3. SECTION OF AORTA WITH A PART OF THE JUVENILE (j.w.) CUT AND EXHIBITING NORMAL MEDIAL AND ADVENTITIOUS COAT, EXCEPT FOR FIBROBLASTIC ELEMENT AND A FEW MONONUCLEAR CELLS (PHOTOMICROGRAPH) × 60.

FIG. 4. MAGNIFIED VIEW OF A PART OF FIG. 3 WITH FIBROBLASTIC ELEMENTS AND A FEW MONONUCLEAR CELLS (PHOTOMICROGRAPH) × 130.

The single specimen available was much younger than the specimens studied by Pande *et al.* (1961) from other hosts, and from its shorter duration inside the coat the reactions appeared milder than those described by them.

Onchocerciasis: Incidence of *O. armillata* in Indian buffalo has been recorded by Bhalerao (1935), Baylis (1939) and Bhatia (1960). The morphology of the parasite, as studied from the fragments of worms of both sexes, did not reveal any difference from the account given by Chodnik (1957) except for the smaller size of the spicule and distribution of the caudal papillae in male. According to Chodnik, the left longer spicule measured 0·32 mm. long × 0·022 mm. wide (0·30 mm. long × 0·024 mm. wide in authors' specimen) and the right shorter spicule 0·16 mm. long ×



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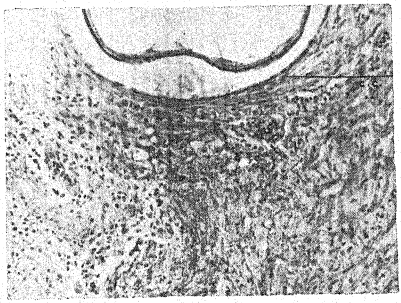

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Onchocerca armillata

FIG. 5. SECTION OF AORTA SHOWING AN ONCHOCERCAL PREADULT TRANSVERSELY CUT WITH ITS UTERUS (ut), WITH EGGS (e) AND INTESTINE (l). NOTE MILD REACTION IN THE VICINITY (CAMERA LUCIDA DRAWING)

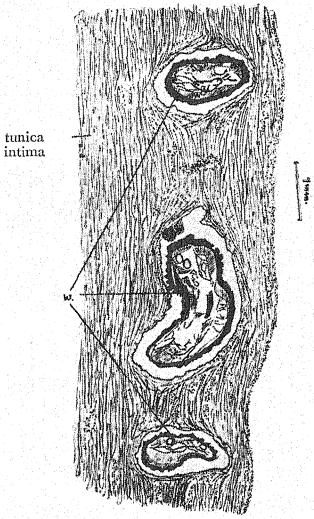
FIG. 6. SECTION OF ONCHOCERCAL NODULE IN AORTA OF BUFFALO-CALF EXHIBITING THICK COLLOGENOUS CAPSULE (c.c.) ROUND THE WORM WITH LYMPHOCYTES, MACROPHAGES AND LARGE NUMBER OF EXTRAVASCULAR BLOOD CELLS (PHOTOMICROGRAPH) × 135.

0·011 mm. wide (0·10 mm. long × 0·016 mm. wide in authors' specimen). The caudal papillae, usually in eight pairs but less frequently in seven pairs, have been stated to be in two groups—the adanal and postanal groups—the former consisting of some which always lie preanally in two pairs immediately lateral to anus. The postanal group, usually consisting of four pairs, had one pair lying close to the end of the tail. The papillae in the specimen studied consisted of two pairs of preanals, one pair of adanal and five pairs of postanals, the last two pairs being smaller in size and situated towards the tip of the tail.

The gross lesions in the form of sinuous galleries were, in one case, associated with a prominent nodule with a centrally reddish zone. In the series of the stained sections the intima around the harboured worms (all preadults) revealed a ring of collogen fibres, forming a thin encapsulation with a few mononuclear cells infiltrated in the vicinity (Fig. 5). The nodulated part, characterized by a thick collogenous capsule in close promixity of worm, revealed grades of necrotic changes and in addition; a diffused haemorrhage with a large number of extra-vascular erythrocytes and fibrin strands was observed. The intimal coat also, with a few necrotic zones, showed a tendency towards calcification in some cases. The area around the worm was riddled with a heavy mononuclear cellular infiltration, predominently with lymphocytes and macrophages (Fig. 6). No pathological changes in the medial and adventitious coats were, however, observed.

Identical gross lesions were also observed in one of the eight caprine cases available for examination and, in the sections, the tunnels harboured preadult worms with similar pathological changes, but of a milder character (Fig. 7). The nodule adjacent to the tunnel, left by the shifting worm, also revealed necrosis but with a centrally calcified mass, and the surrounding tissue was infiltrated with reactionary cells, lymphocytes and macrophages (Fig. 8).

The material from the bullock revealed grossly the entire intimal lining as wavy and irregularly raised over the sinuous galleries occupied by the worms. The prominent nodules with even surface yielded coiled adult male worms from deeper locations of its central portion. Histological study of the lesions with gravid females, cut in different planes, showed the fully formed microfilariae just below the intima. In some other sections, in this series, the gravid females in consequence of their larvae had caused a break in the intimal coat. Chodnik (1958) indicated the position of the microfilariae, both living and dead, in relation to the tunnels and nodules. The manner of escaping of microfilariae after leaving the females has been suggested by him, but in the absence of any evidence he thought that the tunnels, penetrating the aortic lumen, probably terminated in crater-like elevations. He was, however, unable to find mature worms or microfilariae emerging from the openings. The superficial position in the intimal coat invariably observed in case of gravid females points to the possibility of this behaviour in facilitating the escape of microfilarae, whereby a rupture or break in its endothelial lining is caused. Around such females a definite ring of hyalinized tissue with spindle-shaped fibroblast cells was revealed and groups of reactionary cells, mostly lymphocytes with a few eosinophils and macrophages, were seen in the surrounding tissue in addition to cellular debris, with some degree of calcium salt deposits in



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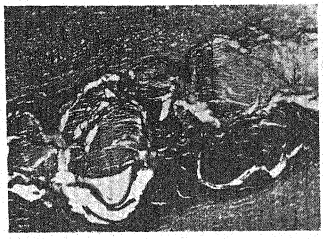

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Onchocerca armillata

FIG. 7. SECTION OF AORTA OF GOAT WITH PREADULT WORM (W) CUT AT THREE PLACES. NOTE MILD REACTION (CAMERA LUCIDA DRAWING)

FIG. 8. SECTION OF CAPRINE AORTA WITH A CENTRALLY NECROSED AND CALCIFIED MASS, INDICATING THE PREVIOUS ABODE OF THE WORM (PHOTOMICROGRAPH) × 50

these tunnels. A definite proliferation of fibro-elastic tissue in these zones was also revealed (Fig. 9).

In case of male worms lying coiled in the centre of the nodules the reactionary zone was surrounded by dead leucocytes and tissue debris along with a large quantity of proliferated fibrous tissue, mostly collogen fibres, in addition to pockets of haemorrhage with prominent reactionary cells, particularly lymphocytes and eosinophils (Figs. 10, 11). These inflammatory reactions, essentially similar to those mentioned by Chodnik as occurring deep in the media, showed no fibrosis, or only a moderate degree of fibrosis in its outer periphery. The authors, however, noticed a definite nodule formation.

The gravid female, occurring in the tunica intima and the endothelial lining of the material from adult buffalo, was similarly associated with a bulging, but without any rupture in its contour. In the endothelium there was a lymphocytic infiltration with the intimal layer all round the worm, exhibiting chronic inflammatory reactions

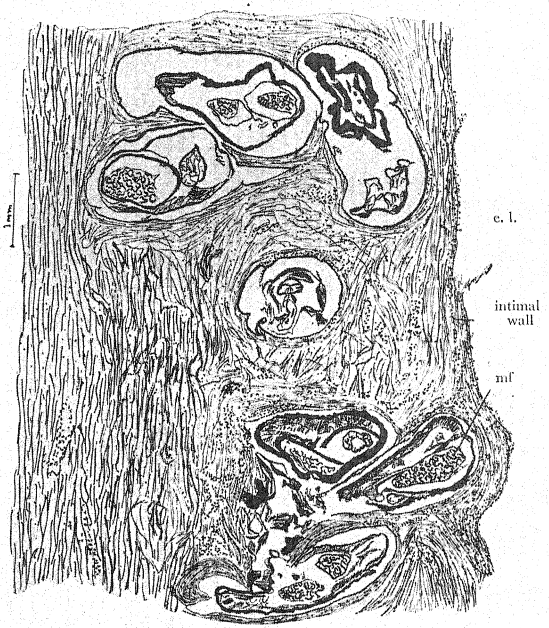
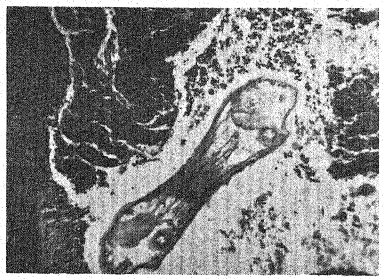


FIG. 9. SECTION OF AORTA OF ADULT CATTLE WITH GRAVID FEMALE HAVING *Onchocerca armillata* MICROFILARIAE (mf). NOTE BULGING OF ENDOTHELIAL LINING (e.l.) AND A BREAK IN THE CONTINUITY OF INTIMAL COAT, WITH PRESENCE OF HYALINIZED TISSUE, FIBROBLAST CELLS, LYMPHOCYTES, FEW EOSINOPHILS AND MACROPHAGES AROUND IT (CAMERA LUCIDA DRAWING)

marked by degenerative changes, and an infiltration of reactionary cells, mostly lymphocytes and macrophages. A slight fibroblastic activity around the worm was also noticed and in the older abodes, in other layers, calcification and an infiltration of lymphocytes, macrophages and a few blood cells in between the fibres was observed (Fig. 12).

The present finding of *Onchocerca armillata* in two of the buffalo-calves, between three to six months in age, with prominent lesions harbouring the preadult stages and characterized by nodulation in addition to sinuous galleries has demonstrated that



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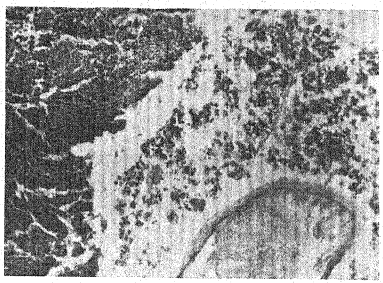

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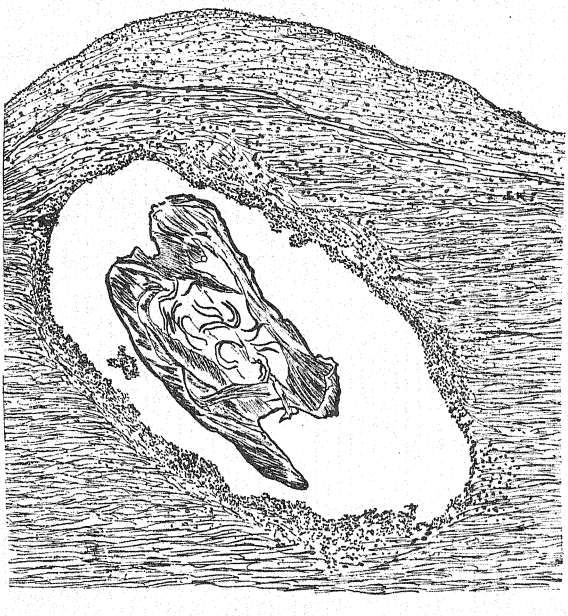
FIG. 10. SECTION OF AORTA OF AN ADULT CATTLE WITH MALE WORM CUT IN THE NODULE. NOTE DEAD LEUCOCYTES AND TISSUE DEBRIS (PHOTOMICROGRAPH) × 60.

FIG. 11. PART OF FIG. 10 REVEALING PORTION OF THE WORM WITH DEBRIS AND SURROUNDING FIBROUS TISSUE WITH CELLULAR INFILTRATION CONTAINING POCKETS OF HAEMORRHAGE (PHOTOMICROGRAPH) × 128.

this parasite is acquired early in life, though the younger calves, one- to three-month old, were free from such lesions. The incidence in this age group, unlike the finding of Patnaik, would be the first record. Another feature with regard to this infection relates to the occurrence in buffalo-calves of nodules in association with preadult female forms, which are marked by a degree of pathological changes that have been described by Chodnik in the nodules associated with adult males in cattle.

The onchocercal material from adult buffalo and bullock exhibited a pathological picture, mainly similar to that given by Chodnik except for the difference that the gravid female was observed to occupy superficial positions, possibly to deposit their microfilariae.

The aortic material from goat (over one year in age), on the other hand, was found to harbour the preadult stages only inside the tunnels, the intima revealing a mild reaction because of its shorter duration. The medial coat, however, was nodulated



Onchocerca armillata

FIG. 12. SECTION OF AORTA OF AN ADULT BUFFALO WITH GRAVID FEMALE CUT, AND REVEALING SIMILAR ENDOTHELIAL BULGING, DEGENERATION AND MONOCELLULAR INFILTRATION (CAMERA LUCIDA DRAWING)

and exhibited profound pathological changes from necrosis to calcification and an infiltration with reactionary cells at such site, the earlier abode of parasite.

SUMMARY

The finding of juvenile stages of the oesophagostomal tumour-worm of dog, *Spirocerca lupi*, in aorta of buffalo-calf and adult buffalo enlarges the range of its known hosts. The lesions in *Onchocerca armillata* infection in buffalo-calves in the age group of three to six months, reported for the first time, have also been studied histologically, and the pathological picture compared with the lesions encountered in adult buffalo, bullock and goat. *O. armillata* appears to establish early in the life of buffalo.

ACKNOWLEDGEMENTS

Thanks are due to the Principal of the College for the facilities provided.

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STUDIES ON BLACKQUARTER

I. ISOLATION AND STUDY OF THE CAUSAL AGENT

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BLACKQUARTER is one of the major diseases of cattle in some of the important cattle-breeding tracts in India. It has been considered by some authors to be a clinical than a bacteriological entity. Kitt (1911) was perhaps the first to suggest that more than one type of clostridium was responsible for the disease. Leclainche *et al.* (1924) expressed the view that the disease was not a single bacteriological entity, but was in this respect similar to gas gangrene in human beings. In India, Edwards (1927) observed that veritable outbreaks of blackquarter were caused by true blackquarter bacilli, while sporadic cases, especially those associated with traumatism, might be caused by other organisms of malignant oedema group. Viswanathan (1937) stated that in Madras blackleg group and *vibrion septique* group of organisms were responsible. Naidu (1938) isolated *Cl. septique* as well as *Cl. welchii* from an outbreak of a disease in cattle akin to blackquarter. Of the 30 cultures isolated from cases of blackquarter in Madras State, 26 were *Cl. chauvoei*, one *Cl. septicum* and three were mixtures of both the organisms (Iyer, 1952). Smith (1957) stressed the importance of *Cl. chauvoei* in the causation of blackquarter. Roberts (1959) summarized the findings of a number of workers in different parts of the world. Results of study on clostridia isolated from a number of cases of blackquarter in different parts of India are presented here.

MATERIAL AND METHODS

Collection of material from cases of blackquarter: Collections for the study of the aetiology of the disease were made personally in the endemic areas during the outbreak season and through the agency of field veterinary officers of different states. The material consisted of affected muscle bits from animals suspected to have died of blackquarter either air dried or preserved in 50 per cent glycerine saline or of aspirated fluid from crepitating swellings in the ailing animals.

Isolation of cultures: This was done by inoculating guinea-pigs, intramuscularly in the thigh region with 0·5 ml. of a 10 per cent suspension of muscle pieces heated at 70°C for 20 minutes mixed with 0·5 ml. of a sterile 5 per cent solution of calcium chloride. Aspirated fluid from blackquarter lesion was injected similarly in guinea-pigs after diluting with normal saline solution. The infected guinea-pigs were invariably destroyed *in extremis*. In most cases guinea-pigs were sacrificed in about 24 hours, while in some death occurred even within 18 hours and a few survived for four to five days. Whenever the guinea-pigs did not die, unheated muscle suspension was injected into fresh lot of guinea-pigs.

Cultures were made from heart blood and from the local lesion of the guinea-pigs in Robertson's cooked meat medium or in brain medium. Smears made from local lesion, heart blood and peritoneal surface of liver (impression) were examined after staining by Gram's method. Nature and extent of lesion were also noted.

Purification of cultures were effected by growing the organisms alternatively in shake cultures (liver infusion agar with 0·5 per cent glucose and 0·05 per cent cysteine hydrochloride) and on blood agar plates. Some of the freshly isolated strains of *Cl. chauvoei* yielded surface growth only after two or three passages in cooked meat medium. One hundred and eighty-eight cultures of clostridia isolated from field cases of blackquarter were studied.

Identification of the cultures: The criteria used for identification of the organism isolated were: (i) morphology, (ii) staining characters, (iii) growth characteristics in cooked meat medium or in brain medium, in shake agar cultures and on blood agar plates, (iv) action on litmus milk, (v) liquefaction of gelatin, (vi) fermentation of sugars, and (vii) haemolysis of sheep erythrocytes. In addition to these tests, the type of lesions produced in guinea-pigs was also taken into consideration.

Fermentation reaction: Fermentation of dextrose, lactose, maltose, mannite, sucrose and salician was studied. Acid production in individual tubes was tested with B.D.H. capillator set employing bromocresol purple as indicator. A pH of 5·8 or less was indicative of positive fermentation reaction, while in the controls and those in which fermentation had not occurred the pH of the medium remained above 6·8.

Some difficulty was experienced in the initial stages of the work as reproduceable results could not be obtained, especially in the case of *Cl. chauvoei* with certain basal media employed. Four different types of basal media were tried, viz., (i) 1 per cent peptone water containing 10 per cent ox serum with incubation done in anaerobic jar, (ii) sugar-free broth with 10 per cent ox serum and a piece of fresh rabbit kidney in each tube, (iii) sugar-free broth with 10 per cent ox serum and 0·05 per cent cysteine hydrochloride, and (iv) 2 per cent peptone water with 10 per cent ox serum, 0·5 per cent sodium chloride, 0·1 per cent each of ferric sulphate, magnesium sulphate, sodium citrate and sodium thioglycollate (a modification of the medium used by Scott (1928).

For the preparation of media, two and three, nutrient broth was rendered sugar free by growing *Cl. welchii* for a minimum period of four to six hours (necessary for the pH to become constant) by which time all the carbohydrates contained in the medium were utilized. The culture was then autoclaved at 120°C for 30 minutes and filtered through Seitz EK filter pads after adjusting its reaction to pH 7·2.

Twenty-five cultures of *Cl. chauvoei* and five of *Cl. septicum* were tested for their fermentation properties of the six sugars mentioned earlier, using the four types of basal media. While 24 of the 25 *Cl. chauvoei* cultures revealed characteristic fermentation pattern in 48 to 96 hours with the basal medium No. 4, only 4, 13 and 10 cultures gave positive reaction with the basal media 1, 2 and 3 respectively. All the *Cl. speticum* showed characteristic reaction with all the four basal media. However, the reaction was quicker with media 2, 3 and 4. On the basis of these results basal medium No. 4 was adopted for the routine fermentation studies in this investigation. The one culture which did not ferment any sugar even with medium No. 4 showed characteristic reaction when tested after three subcultures in cooked meat medium.

Haemolysis of sheep red blood cells: Grimm (1934) and Eugenia (1934) used haemolysis test for differentiating *Cl. chauvoei* and *Cl. septicum*. To 0·9 ml. of normal saline solution in a small test tube 0·1 ml. of 3 per cent washed sheep red blood cell suspension was added, followed by one loopful of a 48-hour liver-meat infusion broth culture of the *Clostridium* under test. In the case of *Cl. chauvoei* lysis of the cells started within one or two minutes, while cultures of *Cl. septicum* did not produce any haemolysis even after two hours. Initial trials with five stock strains each of *Cl. chauvoei* and *Cl. septicum*, and two each of *Cl. welchii* and *Cl. oedematiens* revealed that under the conditions of the experiment only *Cl. chauvoei* strains produced haemolysis. Addition of glucose to the medium in which the organisms were grown increased the haemolytic activity, while the addition of blood serum depressed this activity. The haemolysis was inactivated when exposed to 56°C for ten minutes or to 0·5 per cent formalin. Acidity of the culture did not influence the haemolytic activity.

RESULTS

The results of typing of 188 cultures of anaerobes (172 from cattle, seven each from buffaloes and sheep, and two from goats) are presented in Table I. As it would be seen, 161 of the cultures were *Cl. chauvoei*, six were *Cl. septicum*, five were *Cl. welchii* and four were mixed cultures of *Cl. chauvoei* and *Cl. septicum*. Whenever organisms other than *Cl. chauvoei* were identified, the original guinea-pig cultures were subjected to various tests and the presence of mixed cultures was recognized.

Of the 161 cultures of *Cl. chauvoei* tested, 150 produced haemolysis of sheep erythrocytes, 74 produced liquefied gelatin in six to seven days, and 80 produced slight activity in litmus milk.

All the six cultures of *Cl. welchii* isolated proved to be of type A as ascertained by toxin-antitoxin neutralization test.

Role of Cl. septicum *and* Cl. welchii *in experimental* Cl. chauvoei *infection in guinea-pigs:* Two batches of guinea-pigs were infected intramuscularly with pure culture of *Cl. chauvoei*. Soon after death the animals in one of the groups were kept in a dry bacteriological incubator at 30°C and those of the other groups were left at room temperature (18°–20°C). Post-mortem examination of the animals was conducted at varying intervals and cultures were made from heart blood and local lesion. Results of the tests are presented in Table II.

It would be observed that when guinea-pig carcasses were kept at 18–20°C with an atmospheric humidity of 83 per cent the muscles at the site of inoculation were contaminated with *Cl. septicum* within 24 hours and heart blood in 48 hours. In 72 hours contamination with *Cl. welchii* also occurred.

When the carcasses were kept at 30°C contamination with *Cl. septicum* occurred in 12 hours and with *Cl. welchii* in 20 hours.

DISCUSSION

Difficulties experienced in carrying out fermentation studies of clostridia isolated from cases of blackquarter in the initial stages were overcome by using a basal medium containing certain salts, serum and sodium thioglycollate.

TABLE I. RESULTS OF TYPING OF CLOSTRIDIA ISOLATED FROM CASES OF BLACKQUARTER

States	Species of animals	No. of cultures isolated	Results of typing			
			Cl. chauvoei	*Cl. septicum*	*Cl. welchii*	*Cl chauvoei* and *Cl. septicum*
Madras (including Andhra Pradesh)	Cattle	85	78	3	1	3
Bihar	Cattle	25	22	1	1	1
	Buffaloes	1	—	1	—	1
	Sheep	2	—	2	—	—
	Goats	2	—	2	—	—
Bombay	Cattle	17	17	—	—	—
	Buffaloes	4	2	2	—	—
	Sheep	1	—	—	1	—
Hyderabad	Cattle	18	18	—	—	—
	Buffaloes	2	2	—	—	—
West Bengal	Cattle	11	8	2	1	—
Uttar Pradesh	Cattle	7	6	—	1	—
	Sheep	4	—	4	—	—
East Punjab	Cattle	5	4	—	—	—
Kashmir	Cattle	2	2	—	—	—
Madhya Pradesh	Cattle	1	1	—	—	—
Mysore	Cattle	1	1	—	—	—
Total	Cattle	172	157	6	5	4
	Buffaloes	7	4	3	—	—
	Sheep	7	—	6	1	—
	Goats	2	—	2	—	—

TABLE II. CHARACTERISTICS OF ORGANISMS ISOLATED FROM GUINEA-PIGS AT VARYING INTERVALS AFTER DEATH DUE TO ARTIFICIAL INFECTION WITH *Cl. Chauvoei*

Storage temperature	Interval between death and post-mortem (hr.)	Presence of filamentous forms in liver impression smear	Source of culture	Litmus milk	Gelatin liquefaction	Haemolysis sheep r.b.c.	Dextrose	Lactose	Maltose	Mannite	Sucrose	Salicin	Contaminating organism
18°–20°C	24	—	H.B.	No change	—	+	+	+	+	—	+	—	Nil
	24	+	M	Acid	+	—	+	+	+	—	+	+	*Cl. sepcticum*
	48	+	H.B.	,,	+	—	+	+	+	—	+	+	,,
	48		M	,,	+	—	+	+	+	—	+	+	,,
	72	+	H.B.	Stormy fermentation	+	—	+	+	+	—	+	+	*Cl. septicum* & *Cl. welchii*
	72		M	,,	+	—	+	+	+	—	+	+	,,
30°C	4	—	H.B.	No change	—	+	+	+	+	—	+	—	Nil
			M	,,	—	+	+	+	+	—	+	—	Nil
	6	—	H.B.	,,	—	+	+	+	+	—	+	—	Nil
			M	,,	—	+	+	+	+	—	+	—	Nil
	8	—	H.B.	,,	—	+	+	+	+	—	+	—	Nil
			M	,,	—	+	+	+	+	—	+	—	Nil
	12	+	H.B.	Acid	+	—	+	+	+	—	+	+	*Cl. septicum*
			M	,,	+	—	+	+	+	—	+	+	,,
	16	+	H.B.	,,	+	—	+	+	+	—	+	+	,,
			M	,,	+	—	+	+	+	—	+	+	,,
	20	+	H.B.	Stormy fermentation	+	—	+	+	+	—	+	+	*Cl. septicum* & *Cl. welchii*
			M	,,	+	—	+	+	+	—	+	+	,,

H.B.—Heart blood M— Muscle

It is noteworthy that out of 179 cultures of clostridia isolated from cases of blackquarter in cattle and buffaloes, 161 (90 per cent) were *Cl. chauvoei*, nine (five per cent) *Cl. septicum*, five (2·8 per cent) *Cl. welchii*, and four (2·2 per cent) mixtures of *Cl. chauvoei* and *Cl. septicum*. During the course of this investigation material from three cases of blackquarter in a village were collected on a particular day. Of these, two were collected whithin one hour of death while the third was from one that had died about 18 hours earlier. While *Cl. chauvoei* was isolated from former two *Cl. septicum* was isolated from the latter case. In a cow which showed swelling around the vulvar region, no Clostridia could be isolated from the fluid aspirated from the swelling when the animal was alive, but *Cl. welchii* was isolated from the same area 20 hours after its death. *Cl. septicum* or *Cl. welchii* were isolated from seven more cases in which material for bacteriological examination was collected 15 to 24 hours after death of the animals. It may be of interest to note that whenever material from cases of blackquarter in cattle was collected from ailing animals or soon after their death only *Cl. chauvoei* was isolated.

From the evidence presented here the authors are of the view that only *Cl. chauvoei* is responsible for blackquarter in cattle in India and organisms like *Cl. septicum* and *Cl. welchii* are only post-mortem contaminants. This endorses the view expressed by Ryff and Lee (1946).

Blackquarter in sheep and goats does not appear to be a serious problem in this country, as judged by the response from field veterinary officers for the supply of material from affected cases. From two sheep showing symptoms of blackquarter only *Cl. septicum* was isolated during life as well as soon after death of the animals.

SUMMARY

Of the 179 cultures of clostridia isolated from natural cases of blackquarter in cattle and buffaloes, 161 (90 per cent) were found to be *Cl. chauvoei*, nine (five per cent) *Cl. septicum*, five (2·8 per cent) *Cl. welchii* and four (2·2 per cent) mixed culture of *Cl. chauvoei* and *Cl. septicum*. Of the nine cultures isolated from sheep and goats, eight proved to be *Cl. septicum* and one *Cl. welchii*. *Cl. septicum* and *Cl. welchii* were isolated from guinea-pigs that had died after infection with pure culture of *Cl. chauvoei* when their carcasses were kept for varying periods at 18°—20°C and 30°C.

A basal medium capable of supporting growth of even freshly isolated strains of *Cl. chauvoei* for carrying out sugar-fermentation test has been described.

From the evidence collected it is concluded that in India *Cl. chauvoei* alone is responsible for blackquarter in cattle.

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The co-operation of the Directors of the departments of Animal Husbandry in the various states and of the number of field veterinary officers in the collection and supply of material for the investigation is highly appreciated and gratefully acknowledged. The authors are also thankful to the Director, I.V.R.I. for providing facilities for the work and to Shri M. S. Jayaraman for his efficient technical assistance.

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STUDIES ON CAMEL BLOOD ANTIGENIC FACTORS DETECTED THROUGH CATTLE BLOOD-GROUP REAGENTS

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HAEMATOLOGICAL and biochemical studies have been made on *Camelus dromedarius* or the one-humped camel found in India and Pakistan. Soni and Aggarwala (1958), and Bhatt and Kohli (1959) Kohli and Bhatt (1959) reported results of haematological studies in camels. Kumar *et al.* (1961) made biochemical studies of camel blood and observed that the Indian camels differed from other animals in the protein and lipid contents of their blood.

Blood-group studies have been made most extensively in cattle and fowls; some other species such as sheep, swine, horses, dogs, rabbits, etc., have also attracted the attention of the blood-group workers. But so far no studies have been reported on the antigenic structure of camel blood. Maskar (1949) prepared camel antisera and used precipitation method for testing camel antisera with sera from several other species of animals and pointed out the usefulness of this test in meat inspection. In a previous paper (Chet Ram *et al.*, 1964) data were presented on the comparison of blood factors of cattle and buffaloes. The results of the attempts to detect antigenic factors in camel blood with cattle blood-group reagents are reported in this paper.

MATERIAL AND METHODS

Blood samples were collected from 100 camels, belonging to the Government Camel Breeding Farm, Bikaner, to be tested with 38 cattle blood-group reagents. Blood samples, which were collected in isotonic citrate solution from the jugular vein, were preserved in the laboratory at 5°C in the refrigerator. The red blood cells were separated and 2·5 per cent suspensions were prepared after washing three times in normal saline solution. The detailed method according to which the haemolytic tests were set up in the plastic cavity blocks with appropriate controls and the way the results were noted have been described in detail in a previous paper (Chet Ram *et al.*, 1964).

RESULTS

Out of 38 cattle reagents used in the haemolytic tests only two, Iz_8 and Iz_{10}, were found reactive with some camel erythrocytes, as shown in Table I.

The Table shows that out of 38 antigenic factors for which the camel blood samples were tested only two factors, identical or very similar to those of cattle, are found in camel blood. A comparison of the occurrence of these two antigenic factors in different species is given in Table II.

TABLE I. OCCURRENCE OF CERTAIN ANTIGENIC FACTORS IN 100 BLOOD SAMPLES FROM CAMELS TESTED WITH CATTLE BLOOD-TYPING REAGENTS

Sl. No.	Antigenic factor	No. of animals positive	Sl. No.	Antigenic factor	No. of animals positive
1	A	—	20	Iz_3	—
2	E_1	—	21	Iz_4	—
3	Q	—	22	Iz_5	—
4	G	—	23	Iz_6	—
5	K	—	24	Iz_7	—
6	W	—	25	Iz_8	31
7	R	—	26	Iz_9	—
8	X_1	—	27	Iz_{10}	58
9	V	—	28	Iz_{11}	—
10	J	—	29	Iz_{12}	—
11	L	—	30	Iz_{13}	—
12	M	—	31	Iz_{14}	—
13	Z′	—	32	Iz_{15}	—
14	U_1	—	33	Iz_{16}	—
15	K′	—	34	Iz_{17}	—
16	BF_2	—	35	Iz_{18}	—
17	BF_4	—	36	Iz_{19}	—
18	Iz_1	—	37	Iz_{21}	—
19	Iz_2	—	38	Iz_{22}	—

TABLE II. COMPARISON OF THE ANTIGENIC FACTORS FOUND IN CAMELS WITH OTHER SPECIES

Antigenic factor	No. of animals	Camels	Cattle	Buffaloes	Sheep	Goat	X^2
Iz_8	Having the factor	31	75	121	Not present	Not present	64·7**
	Not having the factor	69	75	29			
Iz_{10}	Having the factor	58	40	Not present	49	50	107·8**
	Not having the factor	42	110		1		

**Highly significant

It will be seen from Table II that factor Iz_8 is absent in sheep and goats but present in other species, whereas factor Iz_{10} is absent in buffaloes and present in other species.

Natural antibodies: Camel normal sera were tested with washed erythrocytes from 20 cattle to find out the presence of any natural antibody in the sera against cattle cells. It was observed that out of 100 camel normal sera tested, 49 caused haemolysis of one or more types of cattle cells. On the basis of these reactions the camel sera could be divided roughly into three groups:

(i) Three sera caused haemolysis of all the 20 types of cattle cells used.

(ii) Forty-six sera haemolysed only some types of cells.

(iii) The remaining 51 sera caused no haemolysis of any of the cattle cells tested.

As all the sera were exhausted, no further absorption and fractionation could be attempted.

Previously 96 camel normal sera were also tested with camel erythrocytes to find out the presence of any natural iso-antibodies. No antibody could be detected in any of the sera tested. It is interesting to note in this connection that factor J or J-like substance, which was found to occur in several other species like cattle, buffaloes, sheep and goats was found to be absent in camel erythrocytes.

SUMMARY

Blood samples from 100 camels were tested with 38 cattle blood-group reagents. The occurrence and frequency of two antigenic factors found common in cattle and camel blood were compared with some other species. Natural antibodies against cattle erythrocytes were found to occur in camel normal sera.

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RELATIONSHIP OF CERTAIN PELT CHARACTERISTICS WITH 'CANARY COLOURATION' OF WOOL

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It has been reported earlier (Nagarcenkar and Bhattacharya, 1964) that the 'Canary Colouration' of wool was due to the colouring pigments in the suint secretions of the sheep. In the present paper, its relationship with certain pelt characteristics has been described.

MATERIAL AND METHODS

These studies were based on the Bikaneri sheep of Chokla type (36 ewes and 12 rams) described earlier (Nagarcenkar and Bhattacharya, 1964). Intensity of discolouration was determined as per the practicable score-card method also described therein.

Staple length of wool: This was determined with the tied wool locks from the 12 different regions as described earlier (Nagarcenkar and Bhattacharya, 1964). These locks were collected at the time of shearing. The staple length was measured by keeping the tied wool lock on a black velvet board against a steel ruler. The length was measured from the base to the tip of the lock accurate to 0·5 cm.

Measurements of fleece density: Estimation of this was made with the help of a small density block according to the method of Ramachandran and Yeri (1954). The density determinations were made on an area of 4 sq. cm. from the 12 regions in the vicinity of the tied wool locks on the right side of all the ewes.

Determination of pH of the suint: This was done according to the method described by Serra and DeMatos (1951). Raw wool samples obtained monthly from two randomly selected 4 sq. cm. tattooed patches on the left side of each of the rams as described earlier (Nagarcenkar and Bhattacharya, 1964) were utilized. The wool samples from both the square patches were bulked together and thoroughly mixed. The pH estimation was done with the help of a Beckman glass electrode pH meter, Model G. In certain cases samples of raw wool obtained in the months of April, October and November (after the shearings) were smaller than 0·5 gm.; in such cases the glass-distilled water was added in proportionately less quantities to the raw wool samples to maintain the ratio of 1:40 between wool and glass-distilled water.

Monthly variations in the skin thickness of rams: The skin thickness determinations of the two tattooed patches, each 4 sq. cm. in size on the left side of each ram, were made

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after removing the skin snipetts with a biopsy punch and determining the thickness with the help of a Vernier Calipers graduated up to 0·1 mm. Four skin thickness measurements were made on fresh snipetts at planes perpendicular to each other and their diagonals, and the average thickness for each snipett was recorded separately.

RESULTS

Relationship between staple length and intensity of discolouration of wool in the ewes and the rams: The data on the staple length of the tied wool locks from the 12 body regions of 11 ewes (belonging to the Control group) and 11 rams were recorded. From this the average staple length of each animal was calculated and the correlation co-efficient values between the two traits were worked out for both the ewes and the rams separately. The data are presented in Table I.

TABLE I. STAPLE LENGTH AND DISCOLOURATION SCORE OF WOOL

Ewes			Rams		
Tattoo No.	Av. staple length (cm.)	Av. discolouration score (units)	Tattoo No.	Av. staple length (cm.)	Av. discolouration score (units)
102	12·875	8·250	81	11·208	8·333
110	10·333	7·750	103	11·417	8·166
117	9·500	7·833	221	12·875	8·667
141	12·333	8·667	229	14·083	7·417
152	9·958	8·083	244	12·542	8·083
170	8·958	8·083	245	11·583	7·583
200	8·542	7·250	251	12·125	8·083
215	8·542	7·833	261	11·375	8·583
231	12·917	6·917	264	11·333	6·000
248	9·792	7·333	266	11·375	7·250
249	9·208	7·000	270	11·333	7·667

The correlation values were 0·23 and 0·11 in case of ewes and rams, respectively, which were both not significant statistically.

Relationship between fleece density and discolouration score of wool: The fleece density on each of the 12 body regions of all the ewes was determined. From these observations, the average density of wool fibres per cm.^{2} of body area was calculated. However, as the ewes were divided into three groups, receiving different photoperiodic treatments which were found to influence the intensity of discolouration of wool (Nagarcenkar and Bhattacharya, 1964) only the data on the 11 ewes belonging to the Control group maintained under normal climatic conditions, have been considered for this study. The same are given in Table II.

TABLE II. FLEECE DENSITY AND DISCOLOURATION SCORE OF WOOL IN THE EWES

Tattoo No.	Av. fleece density (fibres/cm.^{2})	Av. discolouration score (units)
102	694	8·250
110	1,234	7·750
117	1,112	7·833
141	954	8·667
152	924	8·083
170	900	8·083
200	1,326	7·250
215	1,021	7·833
231	1,394	6·917
248	1,243	7·333
249	983	7·000

The correlation co-efficient (r) between the average fleece density and the average discolouration score was found to be $-0·698$, which was statistically significant ($P<0·05$); this means that the intensity of discolouration is linearly and negatively correlated with the fleece density.

pH of suint: Data on only four pairs of rams ('Sun' and 'Shade' groups) were available throughout the year. These are represented graphically in Fig. 1. As reported earlier (Nagarcenkar and Bhattacharya, 1964) the rams received two different treatments—exposure to Sun's direct radiation and continued exposure to shade after preliminary period of three months during which all the rams were maintained in the

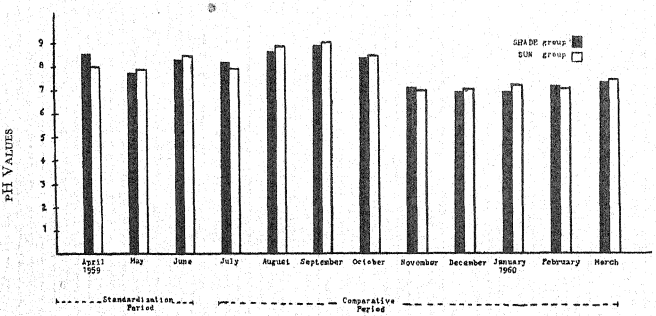

FIG. 1. pH OF SUINT OF MONTHLY WOOL SAMPLES FROM RAMS

shade. The data on the nine months' comparative period were, therefore, subjected to statistical analysis to investigate the effect of this treatment on the pH of suint obtained from the aqueous extracts of the raw fleece samples. The summary of analysis of variance is given in Table III.

TABLE III. ANALYSIS OF VARIANCE FOR THE pH OF THE SUINT FROM THE RAMS DURING THE COMPARATIVE PERIOD

Source of variation	D.F.	M.S.	F. ratio
Pairs	3	0·759	6·658**
Months	8	4·923	43·184***
Treatments	1	0·050	N.S.
Pairs × Treatments	3	0·515	4·51_*
Months × Treatments	8	0·040	N.S.
Pairs × Months	24	0·364	3·193**
Error	24	0·114	

N. S. = Not significant
P** $<$0·01
* P$<$0·05
*** P$<$0·001

On testing the variance due to all other components against the error variance it was seen that the treatment of exposure to Sun's direct radiation had no effect on the pH of the suint of the wool samples collected monthly from the side regions of the rams, whereas significant variation was found due to pairs (animals), months and interactions—pairs × treatments and pairs × months. On further testing the variance due to months, against the variances due to interactions of pairs × months and months × treatments the F values found were 13·525 and 123·075, respectively, both of which were statistically significant at 0·1 per cent level. It can, therefore, be concluded that the main cause of variation in the pH of suint was due to months, i.e., due to the effects of climatic conditions. There was also some variation due to the pairs, i.e., variation between the animals.

As the treatments had no effect, the data on the pH of the suint for all the 12 months irrespective of the treatments were pooled together and analysed statistically. The summary of analysis of variance is presented in Table IV.

TABLE IV. ANALYSIS OF VARIANCE FOR THE pH OF SUINT THROUGHOUT THE YEAR

Source of variation	D.F.	M.S.	F ratio
Pairs	3	0·551	3·800*
Months	11	4·003	27·607***
Pairs × Months	33	0·326	2·248***
Residual (error)	48	0·145	

* P$<$0·05
*** P$<$0·001

On testing the variance due to pairs, months and their interaction against the error variance it was found that the difference due to pairs was significant at 5 per cent level and the differences due to months, and the interaction between pairs ×

months were both highly significant ($P<0.001$). On further testing the variance due to pairs and months against the interaction variance, it was seen that the F value 1·69 for the former was not significant; however, the F value 12·279 for the latter was highly significant at 0·1 per cent level. It is thus proved conclusively that there were monthly variations in the pH of the suint. The data were hence subjected to further statistical analysis for individual monthly comparisons and are presented in ascending order in Table V together with the critical difference values at different levels of significance.

TABLE V. MONTHLY VARIATION IN THE pH OF SUINT

Month	pH value of suint	C. D. values
December	6·919	
January	6·988	
November	7·013	At 5 % level 0·382
February	7·075	At 1% level 0·511
March	7·291	At 0·1% level 0·666
May	7·769	
July	8·003	
April	8·238	
June	8·316	
October	8·316	
August	8·684	
September	8·856	

Differences between the values under the same bar are not statistically significant.

Thickness of skin in the rams: The data on the thickness of skin snipetts based on the same four pairs of rams are presented graphically in Fig. 2. The data for the

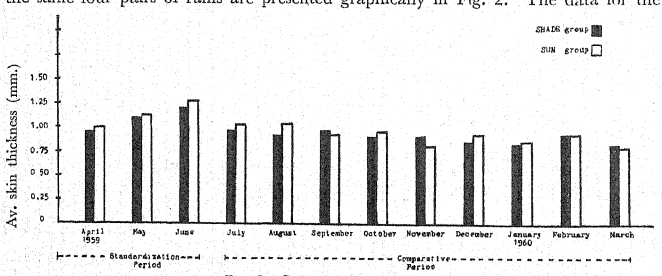

FIG. 2. SKIN THICKNESS IN RAMS

nine months' comparative period when one group of rams received exposure to Sun's direct radiation, were first subjected to statistical analysis. The summary of analysis of variance is presented in Table VI.

TABLE VI. ANALYSIS OF VARIANCE FOR THE DATA ON THE SKIN THICKNESS IN RAMS DUE TO THE TREATMENT OF EXPOSURE TO SUN'S DIRECT RADIATION

Source of variation	D.F.	M.S.	F ratio
Pairs	3	0·027	3·375*
Months	8	0·067	8·375***
Treatments	1	0·007	N.S.
Pairs × Treatments	3	0·046	5·750***
Months × Treatments	8	0·016	2·000 N.S.
Pairs × Months	24	0·021	2·625***
Error	96	0·008	

N. S. = Not significant * $P<0·05$ *** $P<0·001$

On testing the variance due to pairs, months and treatments against the error variance, it was found that the difference due to pairs was significant at 5 per cent level; the difference due to months was significant at 0·1 per cent level and the difference due to the treatments was not significant statistically. Similarly the F values for the interactions of pairs × months and pairs × treatments were found to be highly significant ($P<0·001$), whereas the F value for the interaction of months × treatments was not significant. On further testing the variance due to months against the interaction variances of pairs × months and months × treatments, the F values found were 3·190 and 4·188, respectively, which were both significant at 5 per cent level. It is, therefore, proved that the treatment of exposure to Sun's direct radiation had no effect on the thickness of skin from the side regions of the rams. There was some individual variation in the animals (pairs), and there was variation in the thickness of skin snipetts sampled every month. Also, there were significant interactions between pairs × months and pairs × treatments.

As no effect of the treatment was found, the data on the four pairs of rams throughout the year were pooled together and further analysed statistically. The summary of the analysis of variance is presented in Table VII.

TABLE VII. ANALYSIS OF VARIANCE OF THE DATA ON THE THICKNESS OF SKIN IN RAMS

Source of variation	D.F.	M.S.	F. ratio
Pairs	3	0·057	5·182**
Months	11	0·237	21·545***
Pairs × Months	33	0·017	1·545**
Residual (error)	144	0·011	

** $P<0·01$ *** $P<0·001$

On testing the variance due to pairs, months and their interaction against the error variance, it was seen that there was highly significant variation due to pairs

(animals), months and interaction pairs × months. On further testing the variance due to pairs and months against their interaction variance it was found that the F ratios were 3·353 and 13·941, which were significant at 5 and 0·1 per cent levels respectively. It was thus proved conclusively that there was significant monthly variation in the thickness of skin from the side region of the rams and that there was individual variation in the animals (pairs) and also that the interaction between pairs × months was significant.

Further test was undertaken to find the differences between individual monthly averages. The data in the ascending order are presented in Table VIII together with the critical difference values.

TABLE VIII. AVERAGE SKIN THICKNESS FROM THE SIDE REGION IN THE RAMS

Month of sampling	Average skin thickness (mm.)	C. D. values
March	0·803	
January	0·838	
November	0·863	
December	0·881	
February	0·925	At 5 per cent level 0·073
October	0·931	At 1 per cent level 0·095
September	0·947	
August	0·972	At 0·1 per cent level 0·112
April	0·981	
July	1·000	
May	1·116	
June	1·241	

Differences between the values under the same bar are not statistically significant.

Relationship between the intensity of discolouration of wool and the pH of suint: The correlation co-efficient (*r*) between the average monthly values of the pH of suint and average monthly values of the discolouration score of the wool samples collected from the same sites in all the 11 rams was calculated. The *r* value 0·8397 was found to be highly significant ($P < 0.001$). The regression of the intensity of discolouration of wool on the pH of suint was also calculated from their respective monthly averages. The *b* value was 3·3527. This means that an unit increase in pH of suint resulted in 3·35 units increase in intensity of staining of wool. Regression equation calculated by the method of least squares is given by:

$$Y = 3 \cdot 3527 X - 22 \cdot 4372$$

where,

Y is discolouration score of wool,
and x is pH of the suint.

The significance of the regression was tested by analysis of variance as presented in Table IX.

TABLE IX. ANALYSIS OF VARIANCE TO TEST THE SIGNIFICANCE OF REGRESSION OF DISCOLOURATION SCORE OF WOOL ON THE pH OF SUINT

Source of variation	D.F.	M.S.	F ratio
Due to regression	1	61·837	23·848***
Residual	10	2·593	

*** $P < 0.001$

Relationship between the intensity of discolouration of wool and the skin thickness: The correlation co-efficient (r) between the average monthly values for the thickness of skin from the mid-side region of all the 11 rams and the average discolouration score of the wool samples from these regions was calculated. The r value 0·467 was not statistically significant. It can, therefore, be said that only 21·81 per cent of the variation in the intensity of staining was accounted by the skin thickness of the animals and was linearly related to it.

Relationship between pH of the suint and the skin thickness: Correlation co-efficient (r) between the average monthly values for the pH of suint determined from the aqueous extracts of raw wool samples from the mid-side regions of all the 11 rams, and the average values for the skin thickness from the same regions was calculated. The r value was found to be 0·517, the table value of r at 5 per cent level being 0·576. Thus it is seen that though the correlation co-efficient was not significant statistically, it almost approached significance. It may be stated here that though there are monthly (seasonal) variations in the thickness of skin of sheep, the same could not be very accurately recorded with the help of Vernier Calipers. With better accuracy of measurements with precision instruments it may be possible to find a significant relationship between the skin thickness and the pH of suint. However, from the above data it can be said that 26·73 per cent of the variation in the pH of the suint was linearly related to the thickness of the skin.

DISCUSSION

Lang (1960) stated that staple length was related with discolouration, as he found higher discolouration intensity in animals with two seasons' growth. He mentioned that by arranging shearings at proper time the incidence of canary stain was reduced. Belschner (1957) also made similar observations. However, in the present investigations it was found that there was no relationship between intensity of staining and staple length of wool, and between discolouration intensity and shearing in different months in autumn (Nagarcenkar and Bhattacharya, 1964).

The results obtained have shown a significant negative correlation between the fleece density and intensity of discolouration. The correlation co-efficient value was -0.698 ($P<0.05$) which means, about 49 per cent of the variation in the intensity of discolouration was dependent on the fleece density, and with increase in fleece density the staining was less intense. The reason for this may be that, with high fleece density the ratio of suint/wax was reduced as there would be more number of sebaceous glands to sweat glands per unit area. The studies on fleece rot have also shown that higher wax content in the fleece tends to make the fleece resistant to rot (Hayman, 1953; Thompson, 1953; Fraser, 1957). Marston (1955) stated that composition of the yolk and the amount of it present in a fleece were determined primarily by heredity of the sheep and could be influenced by nutritional and physical environments. Speakman and McMahon (1939) reported less dense fleeces to be more susceptible to weathering. Hewitt (1950) and Morley (1957) suggested that the fleece rot could be reduced by selection. Similarly, from the results presented herein, there seems to be a tremendous possibility of selection for reduction in incidence of 'Canary Colouration' in Bikaneri wools which will be automatically achieved by selection for higher fleece density. It is well known that the fleece density is a highly heritable character (Morley, 1957). Thus by selecting for higher density a two-fold objective of higher fleece yield and reduction of incidence of discolouration in wool can be achieved. The primary follicles in sheep have sweat glands accompanying them and also the largest sebaceous glands. The secondary follicles have only sebaceous glands associated with them. With higher fleece density, the ratio of secondary follicles to primaries (s/p) will be wider and so also the ratio of sebaceous/sweat glands. The latter will in turn affect the composition of the yolk. The quantity of suint in the yolk will be reduced and the quantity of wool fat will be increased. If the 'Canary Colouration' were due to the pigments secreted through the suint (Nagarcenkar and Bhattacharya, 1964) and also evidenced from the results presented by Lundgren (1955), and Binkley and Jones (1959), then the quantity of it in the total yolk will be appreciably reduced due to dilution. Having had to colour greater bulk of fibres the intensity of discolouration would also be naturally reduced.

Another possible way of reducing the incidence of discolouration would be by selection for lesser thickness of skin on the mid-side region in the autumn months. The skin thickness was found to be associated with the pH of the suint secreted by the animal. Various workers (Hardy and Earle, 1939; Hutchison, 1957; Wodzicka, 1958; Alekseeva *et al.*, 1958) reported on the influence of climatic conditions on the skin thickness in sheep. The pH of the suint was in turn found to be highly associated with the staining intensity. It was found that with increasing pH the intensity of discolouration increased and that about 70·5 per cent of the variation in the discolouration intensity of the wool was accounted by the alkalinity of the suint. Results of Serra and DeMatos (1951) and Serra and Albuquerque (1954) agree with the present findings. The sweating being low in winter, the pH of suint is low and consequently there is lesser or no evident discolouration of wool in this season. The skin thickness was higher in the autumn months, especially so in the months of May, June and July when there was onset of staining (Table VII). From the data on pH of suint (Table V) it can be seen that the highest alkalinity of suint was in August and September.

It was reported earlier (Nagarcenkar and Bhattacharya, 1964) that the maximum discolouration score was obtained in the months of July, August and September. Thus some relationship between these three characters is established.

Narayan (1960) reported variation in the follicle population in Chokla, Malpura and Marwari types of Bikaneri sheep. Carter and Dowling (1954) proved that not only the number of sweat glands on the body of sheep was important but also the activity of these glands. They found the individual gland in Lincoln sheep to be six times more active than that in the Merino sheep. Comparative studies in various types of Bikaneri sheep may help in understanding the problem better.

From the results presented here and the above discussion it seems that there are possibilities for selection of types among the Bikaneri sheep which would be resistant to 'Canary Colouration'. Even in the Chokla type, which is by far the best of all the Bikaneri sheep, selection potentialities for reduction of discolouration are ample and could be easily achieved by selection for higher fleece density.

SUMMARY

The alkalinity of suint in Chokla type Bikaneri sheep was seen to increase in autumn months when the discolouration was highest and some evidence was obtained about positive correlation of this with the thickness of skin. Further investigations would be needed to study the size, volume and the sweating activity of the sweat glands in different seasons in the Bikaneri sheep and their relationship with 'Canary Colouration'.

The correlation co-efficient between pH of suint and discolouration score was found to be 0·8397, which is highly significant ($P<0.001$). Regression equation fitted by the method of least squares between the values of pH of suint and discolouration score of wool was:

$$Y=3\cdot3527\times -22\cdot4372$$

where,

Y is discolouration score of wool,

and $\times$ is pH of the suint.

The regression of pH of suint on staining intensity was found to be highly significant ($P<0\cdot001$).

Relationship between staple length and the intensity of discolouration was not statistically significant. A significant negative relationship was found between average fleece density and average discolouration score. The correlation co-efficient value was found to be $-0\cdot698$ ($P<0\cdot05$). The discolouration seems to be due to hereditary factors which are exhibited in congenial climatic conditions of high temperature and humidity. As the intensity of discolouration was found to be associated with fleece density and since the latter is a highly heritable character, there unfolds a possibility of selection of families within the different types of Bikaneri sheep, against the incidence of 'Canary Colouration'. This would achieve a two-fold objective of increasing the fleece weight and also improving the wool quality (regarding colour) which will eventually result in higher profits.

ACKNOWLEDGEMENTS

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ABSTRACTS

Field Trials Using Systemic Insecticides for the Control of Warble on Cattle (*Hypoderma* spp.). WOOD, J. C., BROWN, R. R. M., RICHARDS, M. A. and SPARROW, W. B. 1962. *Vet. Rec.* **74**(5): 130-34.

The results of field trials carried out in Great Britain between the years 1957 and 1961 with two newer organo-phosphorus insecticides, viz., coumaphos (Bayer 21/199, asuntol, Co-Ral) and trichlorphon (Bayer L 13/59, neguvon) as systemic larvicides against warble larvae in cattle have been reported in this article.

A single treatment with 2 per cent coumaphos emulsion applied as a back wash during autumn or early winter gave as good as 80 per cent reduction of warbles (warbles normally appear on the backs of cattle between February and June in Great Britain), but lower concentrations were less effective. Similarly, 2 per cent solution of trichlorphon applied as a back wash during the winter months also gave good control, but the best results were obtained when treatments were carried out in spring as soon as warbles were detectable on the back of the animal. A single treatment with 2 per cent trichlorphon solution a back wash at this time not only killed all the second and third instar larvae present but almost completely prevented any further larvae from appearing later in the season. One such treament is believed to be as effective as two or three monthly treatments with derris wash. However, this spring treatment does not prevent damage to the hide during the year in which it is applied, but it is believed that it provides the most efficient means at present available for significantly reducing the warble-fly population.

Coumaphos used as sprays at 0.5 per cent concentration was less effective than when it was used as a back wash. Trichlorphon given orally at the rate of 80 mg. per kg. body-weight gave inconsistent results and at times proved toxic. However, no symptoms of toxicity were ever observed in any of the 250 animals treated with coumaphos and 658 animals treated with trichlorphon when the insecticides were applied externally.—(R.P.C.)

Effects of Wheat Germ Oil on Reproductive Efficiency in Repeat Breeder Cows. MARION, G. B. 1962. *J. Dairy Sci.* **45**(7): 904.

The effect of wheat germ oil on the reproductive performance in repeat breeder dairy cows has been described. Clinically normal cows which had bred twice or more and kept under the same managerial conditions formed the experimental and control groups. Both the groups were inseminated eight hours after being detected in oestrus from the semen of the same bull and by the same operator as far as possible. The experimental group was given 2 oz. of wheat germ oil per week for six weeks in addition to the normal ration. Pooled data showed a highly significant difference in distribution ($D=0.30$, $P<0.001$) for treated and non-treated groups. Since the reproductive performance of cattle kept on normal diets is not increased by vitamin E,

it has been suggested that substances other than vitamin E present in wheat germ oil may be responsible for this efficiency in repeat-breeder cows.—(M.M.R.N.)

Effect of Source of Protein on Zinc Requirement of the Growing Pigs. SMITH, W. H., PLUMLEE, M. P. and BEESON, W. M. 1962. *J. Anim. Sci.* **21:** 399–405.

The authors conducted two experiments to study the effect of source of protein on the utilization of zinc in pigs. In the first experiment three-week-old 40 pigs were divided into five groups. The sources of protein for individual groups were dried skimmed milk, isolated soyabean protein, soyabean oil meal, commercial casein and vitamin-free casein. Feeding was continued for five weeks. The pigs receiving milk protein containing 6–18 p.p.m. zinc showed the maximum growth-rate, being 0·67 lb. per day. They did not show any symptoms of zinc-deficiency. The growth rate with soyabean meal protein, vitamin-free casein, commercial casein and isolated soyabean-meal protein ration with 22, 6, 18, and 16 p.p.m. of zinc showed growth-rate of 0·27, 0·47, 0·46 and 0·13 lb. per day respectively. Pigs receiving milk protein rations did not show any symptoms of zinc-deficiency, while those receiving soyabean protein showed parakeratosis. Feed requirements per pound of gain in body weight were higher in soyabean-meal group. After five weeks each of the five groups was divided into two groups. One group was maintained on the original ration and the other group was supplemented with zinc at the rate of 50 p.p.m. zinc as zinc oxide. Pigs maintained on soyabean ration showing the zinc-deficiency symptoms were relieved by above supplementation. Seven perakeratotic pigs were divided into two groups of four and three each. The four pigs fed on dried skimmed milk without supplementation of zinc showed a marked improvement after 21 days of feeding. Other group of three supplemented with 125 p.p.m. of copper to basal diet did not show any improvement. In another experiment three-week-old 30 pigs were divided into five lots. First lot was maintained on soyabean oil-meal basal ration and second with supplementation of 50 p.p.m. zinc as zinc oxide. The third lot with basal ration having 30 per cent lactose substituted for equal parts of cerelose and corn starch. Lot 4 basal ration with soyabean oil-meal protein autoclaved for one hour at 230° F and Lot 5 same as Lot 1, except that the metal pens were lined with wood. Supplementation of zinc to soyabean oil meal increased the growth-rate and feed consumption. Autoclaving of protein and supplementation with lactose had no effect. The feed required per pound of grain was highest in the lot maintained in pens lined with wood and lowest in the zinc-supplemented lot.

Perakeratotic pigs fed 50 p.p.m. zinc 450 p.p.m. EDTA (Ethylene dinitrilo tetra acetic acid disodium salt) and autoclaved-isolated soyabean protein gained 35, 41 and 42 per cent more than the controls respectively. However, the differences were not statistically significant. The authors concluded that diets containing milk protein as sources of protein stimulated the utilization of zinc resources available in the ration more efficiently than those animals receiving soyabean as source of protein. Addition of zinc at the rate of 50 p.p.m. to the rations of pigs suffering from perakeratosis showed marked improvement.—(B.C.J.)

REVIEW

THE ANATOMY OF THE SHEEP. NEIL D. S. MAY. 1964. 2nd ed., University of Queensland Press, St. Lucia, Brisbane, Queensland. xiii+369, Figs. 77.

Horse has been the type animal for study in the laboratory as well as in the classroom for a veterinary student. With the shift from draught to food animal, in general, the emphasis in the classroom has also been shifting to food animal. In Australia–the land rich in sheep population–attempts are being made to introduce sheep among the main dissection subjects.

The publication *The Anatomy of the Sheep* was started in the form of notes prepared during the process of dissection, with the purpose of making dissection easier for the students. The present edition, in addition to the chapters on 'Trunk' and 'Limbs', which appeared in the first edition too, contains chapters on 'Head and Neck', 'Osteology' and 'Arthrology'.

The body of the animal is divided into six regions, viz., forelimb, thorax, abdomen, hind limb, pelvic cavity and head and neck, and described in chapters separate for each of them in the sequence in which the different parts of the animal should be studied.

The muscles, nerves, arteries, veins and organs present in these regions are dealt with in the order they come across during dissection. The steps to be followed in dissection are given first and then the description of the structures exposed.

Osteology and arthrology have been dealt with in separate chapters and not in the chapters dealing with the respective regions. The 'Comparative Table of Organs of Domestic Animals' is very informative and helps the student to get an idea about the similar organs in different kinds of animals studied. Inclusion of a table giving comparative study of bones, joints, blood vessels, nerves, etc., in various domestic animals would have enhanced the value of the publication further.

The publication mainly intended to serve as a teaching manual does not deal with the subject in detail but this in no way minimizes the value of the book.

The book is well-illustrated. The get up of the publication is good. The binding is excellent. The book is a valuable asset to a veterinarian, whether a student, field worker or research worker.—(R.R.L.)

AUTHOR INDEX

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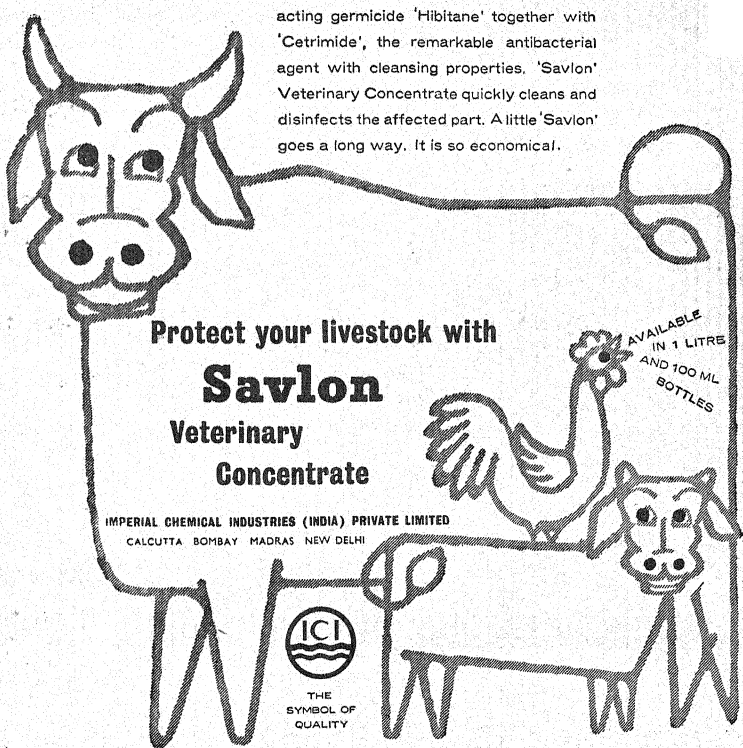

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